# Exploring the genetic and mechanistic basis of resistance to take-all disease in wheat

Submitted by

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## Abstract

Take-all, caused by the soil-borne ascomycete fungus *Gaeumannomyces graminis* var. *tritici*, (*Ggt*), is a root disease that devastates wheat production worldwide. Current control measures consist of partially effective chemical seed dressings and cultural methods such as crop rotation. There is currently no genetic control of the disease.

The first aim of this PhD project was to characterise a range of diploid and hexaploid wheat germplasm that possess a promising level of take-all resistance under field conditions. Both above and below ground phenotyping was carried out and soil moisture probes were used to evaluate upper root function for a range of hexaploid varieties. A diploid *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population was screened and revealed a good spread in susceptibility to take-all across two field seasons. The population has subsequently been genotyped and genetic analyses will be carried out to explore the genetic basis of resistance.

*Phialophora* fungal species, belonging to the genus *Gaeumannomyces*, colonise wheat roots but do not destroy the vascular tissue and have previously been found to suppress take-all disease. In the second approach to control *Ggt,* winter wheat varieties on the AHDB Recommended List (RL) were screened for their ability to build-up natural populations of *Phialophora* fungi in the field. Differences were revealed in their potential to build-up *Phialophora* spp. under a first wheat crop.

A *Phialophora* isolate collection was gathered and draft genomes were sequenced, assembled and annotated for the three *Phialophora* spp. found in UK soils. Preliminary analysis suggests that considerable polymorphism may exist between homologous genes found in all three species. These findings provide a novel contribution to the potential of these two differing control mechanisms against take-all disease.

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## Abbreviation list

| | |
|---|---|
| (R) | resistant |
| (S) | susceptible |
| µg | microgram |
| µl | microlitre |
| µM/mM | micromolar |
| 2,4-DAPG | 2,4-diacetylphloroglucinol |
| AHDB | Agriculture and Horticultural Development Board |
| ANOVA | analysis of variance |
| BLAST | Basic Local Alignment Search Tool |
| bp | base pair |
| BT | back transformed means |
| BUSCO | Benchmarking Universal Single-Copy Orthologs |
| CDS | the coding sequence |
| CTAB | cetyltrimethylammonium bromide |
| d.f. | degrees of freedom |
| DArT | Diversity Array Technology |
| defra | Department for Environment, Food and Rural Affairs |
| DIBOA | 2,4-dihydroxy-1,4-benzoxazin-3-one |
| DIMBOA | 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one |
| EDTA | ethylenediaminetetraacetic acid |
| EI | Earlham Institute |
| e-RA | electronic Rothamsted Archive |
| EU | European Union |
| FEB | Fusarium Ear Blight |
| *g* | G-force |
| GFP | Green Fluorescent Protein |
| *Gga* | *Gaeumannomyces graminis* var. *avenae* |
| *Ggg* | *Gaeumannomyces graminis* var. *graminis* |
| *Ggm* | *Gaeumannomyces graminis* var. *maydis* |
| *Ggt* | *Gaeumannomyces graminis* var. *tritici* |
| GS | growth stage |
| HCl | hydrochloric acid |
| HSE | Health and Safety Executive |
| Hz | hertz |
| INSDC | International Nucleotide Sequence Database Collaboration |
| IPM | integrated pest management |
| ITOL | The Interactive Tree of Life |
| ITS | internal transcribed spacer |
| JIC | John Innes Centre |
| K | exchangeable potassium |
| Kb | kilobase |
| LAMP | loop-mediated isothermal amplification |
| Ltd. | Limited |
| m/s | meter per second |
| MAS | marker assisted selection |

| | |
|---|---|
| Mb | megabase |
| ml | millilitre |
| mm | millimetre |
| mM | millimolar |
| nabim | National Association of British and Irish Millers |
| NCBI | National Centre for Biotechnology Information |
| ng | nanogram |
| NR | not recommended |
| P | plant available phosphorus |
| PacBio | Pacific BioSciences |
| PCR | polymerase chain reaction |
| PDA | potato dextrose agar |
| PGRs | plant growth promoters |
| PSMD | potential soil moisture deficit |
| qPCR | quantitative polymerase chain reaction |
| QRL | quantitative resistance loci |
| QTL | quantitative trait loci |
| rDNA | ribosomal DNA |
| REML | Residual Maximum Likelihood |
| RFLP | restriction fragment length polymorphism |
| RL | Recommended List |
| Rres | Rothamsted Research |
| rRNA | ribosomal ribonucleic acid |
| $r_s$ | Spearman's rank correlation coefficient |
| SARDI | The South Australian Research and Development Institute |
| SDS | Sodium Dodecyl Sulfate |
| SED | standard error of differences |
| SNP | single nucleotide polymorphism |
| sp. | species |
| spp. | species plural |
| SSD | single seed descent |
| TAB | take-all inoculum build-up |
| TAD | take-all decline |
| TAI | take-all index |
| TBE | Tris/Borate/EDTA |
| TE | Tris-EDTA |
| TGAC | The Genome Analysis Centre |
| tRNA | transfer ribonucleic acid |
| UAV | unmanned aerial vehicles |
| UK | United Kingdom |
| USA | United States of America |
| UV | ultraviolet |
| V | voltage |
| var. | variety |
| WGIN | Wheat Genetic Improvement Network |
| WISP | Wheat Improvement Strategic Programme |
| YEP | Young Entrepreneur Programme |

# Chapter 1. General Introduction

## 1.1 Plant pathogens and global food security

The world's population is expected to increase to over 9 billion in 2050 (Gerland et al., 2014). Worldwide, plant pathogens (viruses, bacteria, fungi, oomycetes, nematodes, mollicutes and protozoa) reduce the yields of crops by 10-16% (Strange and Scott, 2005, Oerke, 2006) and, along with land shortage and increased urbanisation, threaten the demand that global food production must be increased by ~50% to feed the world's growing population (Chakraborty and Newton, 2011). Crop losses, from pests and pathogens, are thought to be even higher in developing countries where subsistence agriculture occurs, as it is not always possible to implement control methods (Christou and Twyman, 2004, Lucas, 2011). Of the total wheat harvested in developing countries, 100% is presumed to be used for human consumption, compared to only 45% of total wheat harvest from developed countries (the remainder is used for biofuels and animal feed) (Fisher et al., 2012). Fisher et al. (2012) calculated that a low level of persistent disease resulted in losses that, if they were alleviated, could feed 8.5% of the world's population of 7 billion during 2011.

In addition, climate change is currently increasing some pathogen ranges and triggering the emergence of plant diseases into previously unaffected environments (Anderson et al., 2004, Bebber et al., 2013). The increased global connectivity has also led to the introduction of disease and pests into new regions via trade links (Anderson et al., 2004, Fisher et al., 2012). For example, Chalara dieback of ash (causal agent: *Hymenoscyphus fraxineus*) was introduced into the United Kingdom (UK) via infected trees sent from a nursery in the Netherlands. Regardless of heightened biosecurity measures, there will be a continuation in the spread of invasive pests and pathogens into new geographical regions (Woodward and Boa, 2013).

Following the outbreak of Chalara ash dieback in the UK, the UK Plant Health Risk Register has been initiated and provides monthly reviews of pest and pathogen

threats to UK plant species (https://secure.fera.defra.gov.uk/phiw/riskRegister/). The register highlights the importance of providing tools to allow researchers and the government to prioritise action against plant pathogens. Risks have also been recorded on the register to document concerns over the availability of control options for plant pathogens, such as the reduced availability of methods to control potato blight (*Phytophthora infestans*).

Further challenges exist with providing crop resilience through plant disease management and control methods. Fewer chemical controls are being developed due to increased regulations, such as the EU pesticide legislation Directive 2009/128/EC that aims to provide more sustainable use of pesticides with a reduction in both environmental and public health risks. There are also fewer active ingredients available in the agrochemical toolkit as well as the lack of pesticide selectivity and increased fungicide resistance (Cools and Hammond-Kosack, 2013). Disease control mechanisms therefore need to look beyond chemistry for solutions and it is likely that farmers will begin to demand a higher level, as well as a more durable, crop varietal resistance (Summers and Brown, 2013). However, there are also a number of breakdowns in varietal resistance which are threatening control against plant pathogens in the UK, as well as worldwide.

### 1.2 Plant pathogens of wheat

Wheat is grown on all continents, except Antarctica, and is endlessly challenged by plant pathogens and subsequent diseases. The most important diseases of wheat worldwide are caused by fungi (Singh et al., 2016), whereas diseases caused by oomycetes, bacteria and viruses are considered to have less effect on yield losses (Oerke, 2006).

Both biotrophic and necrotrophic fungi cause significant damage to wheat crops (Singh et al., 2016). Wheat rusts, including yellow rust (*Puccinia striiformis* f. sp. *tritici*), brown rust (*Puccinia triticina*) and stem rust (*Puccinia graminis* f. sp. *tritici*) and powdery mildew (*Blumeria graminis* f. sp. *tritici*) are biotrophic fungi that attack the leaves, stems and ears of living hosts and cause significant yield losses. Wheat rust is present in all wheat producing continents (Fisher et al., 2012) and

although substantial progress has been made in identifying and introgressing plant host resistance, the fungi have shown the ability to rapidly overcome deployed resistance genes (Singh et al., 2016). The emergence of new pathogen races into a region can also threaten deployed resistance genes. The UK Cereal Pathogen Virulence Survey (UKCPVS) (http://www.niab.com/pages/id/316/UKCPVS) has recently reported a new yellow rust race, Kranich, that has been detected in the UK. The current risk of this new race, first identified in Denmark and Sweden, is unknown. Other foliar wheat pathogens include the hemibiotrophic *Zymoseptoria tritici* that causes septoria leaf blotch disease; considered the most important disease of winter wheat in the UK due to substantial crop losses (https://cereals.ahdb.org.uk). *Zymoseptoria tritici* has developed resistance to a number of chemistries and a recent decline in the efficacy of azole fungicides are threatening control methods against the disease (Cools and Fraaije, 2008, Fraaije et al., 2012). Similar breakdowns in resistance have been found for azole fungicides that are the main control method against *Fusarium* spp. that form the species complex of Fusarium Ear Blight (FEB) (Popiel et al., 2016). A number of causal agents of FEB have been identified, including *F. graminearum* and *F. culmorum* that are most commonly found in the UK, as well as *F. poae*, *F. avenaceum*, *F. sporotrichoides* and *Microdochium nivale* (Osborne and Stein, 2007). *Fusarium* spp. produce mycotoxins that pose a health threat to both animals and humans and mycotoxin levels are subsequently monitored in grain and flour.

Wheat fungal pathogens have shown the ability to extend their host range, such as the wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) expanding its host range, multiple times across Europe, to triticale (*Triticosecale*) (Troch et al., 2012). As well as expanding its host range to triticale, the rice blast fungus (*Magnaporthe orzyae*) has spread to wheat and is the causal agent of wheat blast, a disease that devastates wheat crops in South America (Igarashi et al., 1986) and has recently spread to Asia (Callaway, 2016).

Soil-borne fungal pathogens predominately infect the stem and roots of wheat plants and include *Gaeumannomyces graminis* var. *tritici*, the causal agent of take-all disease of wheat and *Oculimacula yallundae* and *O. acuformis* that cause

eyespot. Soil-borne fungal pathogens can persist in the soil, in the absence of the host, for long periods of time through the production of resting structures that include mycelium, spores and sclerotia, presenting difficulties when attempting to control the disease. Agricultural practices can also lead to the persistence of fungal inoculum in the soil, for example spores of wheat stem rust (*P. graminis* f. sp. *tritici*) can overwinter on crop stubble (Fisher et al., 2012) as well as the fungi such as *Fusarium* spp. and the take-all fungus surviving saprotrophically on crop debris, leading to infection of subsequent crops (Cook, 2003).

### 1.3 Wheat cultivation and cultivar improvement

Wheat is the third most extensively produced cereal crop worldwide (http://faostat3.fao.org/) and is a staple food crop for around 40% of the world's population (Kilian et al., 2009). Wheat was first domesticated around 10,000 years ago along with rice and maize as human societies transferred from hunting and gathering to agriculture (Doebley et al., 2006). *Triticum aestivum* L. ($A^uA^uBBDD$) is the most economically important wheat species (Kilian et al., 2009). This hexaploid species is also known as common or bread wheat and is a member of the grass family Poaceae. *Triticum aestivum* consists of three subgenomes, derived from *Triticum uratu* (AA), *Aegilops speltoides* (BB) and the wild goat grass *Aegilops tauschii* (DD) (Petersen et al., 2006, Marcussen et al., 2014). *Triticum aestivum* is the product of a cross between the two A and B genome donors to form a domesticated tetraploid wheat, *Triticum turgidum,* which then hybridised with the D genome donor of *A. tauschii* (Petersen et al., 2006, Marcussen et al., 2014). A chromosome-based draft sequence of the *T. aestivum* genome has recently been produced (Mayer et al., 2014).

Domestication has reduced the genetic diversity of wheat due to genetic bottlenecking from the limited hybridisation events and therefore increased vulnerability to biotic stresses such as pests and plant pathogens (Fu and Somers, 2009). The reduced genetic pool of modern wheat varieties (Valkoun, 2001) and the demand for an increase in wheat yield potentials (Duveiller et al., 2007), from an ever-increasing human population, has led to the development of modern wheat improvement strategies. In order to implement such strategies, it is necessary to

exploit the genetic resources of wild progenitors and wheat relatives. Thus introducing novel traits for disease resistance into modern wheat lines via wide crosses.

Breakdown of disease resistance as well as emerging pathogens and/or races, threaten the farmer's crops and therefore require plant breeding programmes to constantly identify novel sources of plant resistance (McGrann et al., 2014). Research scientists often focus on identifying sources of plant resistance for just one disease, yet farmers need wheat varieties to be resistant to a whole range of pathogens. It is also important to assess for trade-offs between different traits. Disease resistance to powdery mildew in barley (*Hordeum vulgare* L.) is conferred by *mlo* alleles, yet the use of the *mlo* alleles leads to a trade-off with increased susceptibility to a number of other diseases (McGrann et al., 2014), including FEB in wheat (Jansen et al., 2005).

### 1.3.1 UK commercial wheat varieties

Wheat is the main crop produced in the UK (http://faostat3.fao.org/) and in order for a wheat variety to be eligible for commercial cultivation and marketing, it must be added to the UK National List for the main agricultural and vegetable species. Varieties are trialled over a two year period to ensure the variety is distinct, sufficiently uniform and stable and has a satisfactory value for cultivation and use (www.fera.defra.gov.uk). Data collected during trials for the National List is then used to select varieties for the Agriculture and Horticulture Development Board (AHDB) Recommended List (RL) and further independent field trials are carried out. The AHDB RL (cereals.ahdb.org.uk) provides annual information on the yield and performance of cereal varieties to farmers from independent field trials. This allows farmers to decide upon a suitable variety for their region and market. The largest market for wheat is flour production and the National Association of British and Irish Millers (nabim) classify varieties into four groups to indicate use of the grain (www.nabim.org.uk). AHDB assess and evaluate varieties on grain yield stability with and without fungicides and general qualities of the crop such as endosperm texture and percentage of protein content along with information on susceptibility to diseases, pests and lodging. Wheat disease and pest resistance of varieties is

provided for powdery mildew (*B. graminis* f. sp. *tritici*), yellow rust (*P. striiformis* f. sp. *tritici*), brown rust (*P. triticina*), leaf and glume blotch (*Parastagonospora nodorum*), septoria leaf blotch (*Z. tritici*), eyespot (*Oculimacula* spp.), FEB (*Fusarium* spp.) and orange wheat blossom midge (*Sitodiplosis mosellana*). Varietal resistance to septoria leaf blotch and orange wheat blossom midge is currently of high importance (Summers and Brown, 2013), due to the potential of such high yield losses in the UK as well as the developing resistance of *Z. tritici* populations to azole fungicides. The AHDB website also provides information on any breakdown of resistance (cereals.ahdb.org.uk). There is a high turnover of varieties on the AHDB RL in response to resistance breakdown and the subsequent negative effect on potential yields.

### 1.4 Take-all disease of wheat

Take-all is a root disease caused by the soil-borne ascomycete fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*) (previously named *Ophiobolus graminis*) (Walker, 1981), which devastates wheat production worldwide (Huber and McCaybuis, 1993). The name take-all was first described in Australia around 1870 (Hornby et al., 1998) and is now considered the most important root disease of wheat worldwide (Freeman and Ward, 2004). The pathogen also infects the cereals barley, rye and triticale (Rothrock, 1988). The root disease causes the crop to become stunted and the grain prematurely ripens, leading to a loss in both grain quality and potential yield. There is currently no form of genetic control for the disease and control measures predominately consist of crop rotations and partially effective seed dressings. The most recent estimate of an average annual UK yield loss from the disease is 5-20% but is considerably more when the severity of the disease is high, with the annual cost for UK farmers thought to be up to £60m (Take-all in winter wheat-management guidelines, Autumn 2006, AHDB).

#### 1.4.1 The *Gaeumannomyces genus* and the *Gaeumannomyces-Phialophora* complex

*Gaeumannomyces graminis* var. *tritici* (*Ggt*) (Walker, 1972) belongs to the family *Magnaporthaceae* which contains seven species belonging to the *Gaeumannomyces* genus: *G. graminis*, *G. caricis*, *G. cylindrosporus*, *G. incrustans*, *G.*

*medullaris*, *G. amomi* and *G. wongoonoo* (Hornby et al., 1998). Anamorphs of the *Gaeumannomyces* genus, except for *G. medullaris*, are *Phialophora* spp. (Hornby et al., 1998, Freeman and Ward, 2004). The related *G. graminis* varieties and *Phialophora*-like anamorphs, which infect cereals and grasses, make up the *Gaeumannomyces-Phialophora* complex. There are four varieties of *G. graminis*: var. *tritici* (*Ggt*), var. *avenae* (*Gga*) (Dennis, 1960), var. *graminis* (*Ggg*) (Walker, 1972) and var. *maydis* (*Ggm*) (Yao et al., 1992). These have recently been assigned species status (Hernández-Restrepo et al., 2016). Although *Ggt* is pathogenic to barley, rye, triticale and many other grass species, it is the main cause of take-all disease of wheat. *Gaeumannomyces graminis* var. *avenae* infects a number of species within the *Poaceae* family but is the main cause of take-all of oats (Hornby et al., 1998). *Gaeumannomyces graminis* var. *graminis* is considered to be pathogenic on rice, turf grasses and Bermuda grass (Freeman and Ward, 2004) but rarely pathogenic on other cereals (Holden, 1976). *Gaeumannomyces graminis* var. *maydis* was described in China and is pathogenic to maize (Yao et al., 1992), yet studies have revealed this variety also causes slight infections in wheat, rice, barley, sorghum and a number of grasses (Hornby et al., 1998). *Gaeumannomyces graminis* var. *tritici*, *Gga* and *Ggg* all produce hyphopodia (specialised root-infecting structures) during root infection. The varieties within *G. graminis* are homothallic (self-fertile) and differ in the shape and size of ascospores as well as the range and colonisation of host root tissue (Hornby et al., 1998).

### 1.4.2 Disease development and symptoms

The take-all fungus survives saprotrophically on crop debris and this forms the main inoculum, infecting consecutive wheat crops (Cook, 2003). The level of inoculum rapidly declines in the absence of a susceptible crop since *Ggt* is a poor saprotrophic competitor. Therefore, little disease occurs in a first wheat crop after a non-susceptible break crop. However, *Ggt* also survives on volunteer cereals and grasses and this can lead to inoculum in field soil. In the first wheat crop take-all inoculum will begin to build-up in the soil and this can cause severe disease in a second wheat crop. During severe disease years, perithecia, which release ascospores, may develop on stem bases or on harvested stubble. However, as a

source of inoculum, ascospores are thought to have a limited role in initiating infection (Freeman and Ward, 2004). The lowest yield usually occurs after two consecutive wheat crops due to high inoculum levels building up in the soil. The phenomenon of take-all decline (TAD), the natural soil suppression of take-all disease, occurs around the $4^{th}$-$5^{th}$ consecutive wheat crop, reducing the severity of the disease.

For crops at risk of take-all disease primary infection occurs in the autumn when germinating seeds come into contact with take-all inoculum in the soil. Once roots come into contact and infection is initiated, dark runner hyphae grow over the surface (Figure 1.1) and produce hyaline branches that form infection hyphae and penetrate through the root cortex and into the stele. Hyphopodia or penetration pegs develop from the tip of infection hyphae and via mechanical pressure penetrate the cell wall (Skou, 1981). This allows the fungus to invade and destroy root vascular tissue which leads to the formation of black necrotic lesions (Figure 1.1), that have been found to disrupt water and nutrient uptake of the plant (Pillinger et al., 2005). The presence and percentage of roots infected with take-all lesions is used as a measure to assess and compare diseased plants.




Figure 1.1. Take-all infected wheat seedling. Magnification 40.3 X.

Secondary infections occur in spring and summer from root-to-root contact in the soil between infected roots and developing crown roots. A high level of primary infection subsequently leads to a high level of secondary infection. Above-ground take-all symptoms can be seen during severe disease outbreaks in the early summer and consist of general stunting of the plant, premature ripening and poor grain fill visible as 'take-all patches' of bleached and empty ears as well as blackening of stems bases (Figure 1.2) (Freeman and Ward, 2004). Poor grain filling at this stage can result in significant losses in both grain quality and yield (Schoeny et al., 2001). In addition, the damage to the root systems during take-all epidemics can prevent the crop's capacity to uptake nitrogen and can increase the risk of nitrate leaching from the farmer's soil (Macdonald and Gutteridge, 2012). This is a significant environmental impact of severe take-all disease, since nitrate leaching can pollute water sources and cause eutrophication.



**Figure 1.2.** Development of take-all patches across the Rothamsted Farm (Photograph credit: Visual Communications Unit at Rothamsted Research).

### 1.4.3 Take-all field populations

Two genetically different groups of *Ggt* populations have been identified in the field. A number of studies across the world have demonstrated this with varying molecular tools and because of this, differences exist in the naming of the two groups: N/R (O'dell et al., 1992, Bryan et al., 1995), T1/T2 (Bateman et al., 1992), A1/A2 , $G_1/G_2$ (Lebreton et al., 2004) and A/B (Freeman et al., 2005). Recently, Daval et al. (2010) gathered a collection of 98 *Ggt* isolates from across the world to compare two PCR assays used to discriminate $G_1/G_2$ and A/B groups. The study found that there was a strong association between the $G_1$, B, R, T2 as well as between $G_2$, A, A1, N and T1, confirming that two genetically different groups of *Ggt* populations exist, with minor gene flow between the two groups (Daval et al., 2010).

There is evidence that the population structure of *Ggt* can affect take-all epidemics (Bateman et al., 1997). Bateman et al. (1997) also found that population structures of *Ggt* were affected by crop, T1 was more associated with wheat whilst T2 was more associated with barley. Bryan et al. (1999) found that the R isolate group were able to infect rye, whilst the N isolate group were not able to infect rye. Field experiments have shown that repeated application of silthiofam, a seed treatment against take-all, leads to a selection of B type less sensitive isolates (Freeman et al., 2005).

### 1.4.4 Isolation of take-all and species diagnostic methodologies

Traditional isolation methods consist of isolating the take-all fungus from susceptible plants grown as bait in soil. Infected root pieces are surface sterilised for 5 minutes in sodium hypochlorite (1:5 dilution), followed by triple rinsing in sterile distilled water. Root pieces are then placed onto PDA agar plates, amended with antibiotics. Cultures thought to be *Ggt* are transferred to fresh plates and subcultures are further performed. Growth of *Ggt* from the roots usually occurs after 2-4 days and is pale in colouration with curling back of hyphal tips. As cultures age they become darker in colouration ranging from pale grey to black. Cultures can be maintained on agar blocks under sterile distilled water at room temperature for a number of years (Boesewinkel, 1976).

Pathogenicity tests are performed in order to confirm an isolate is *Ggt* via pot bioassays. *Gaeumannomyces graminis* var. *tritici* inoculum can either be dispersed throughout a mixture of sand and soil or an inoculated agar disc can be placed on the soil with seeds placed on top. Pots are then kept in a controlled environment of 15/10°C (day/night temperature, 16 hr day) for five weeks (Hornby et al., 1998). Roots are then examined for the presence of characteristic black necrotic take-all lesions and the percentage of infected roots can be calculated. Microscopy-based diagnosis of spore morphology cannot be carried out for *Ggt,* since the microorganism lacks an active spore state (Okubara et al., 2005). Current molecular diagnostic methods predominately consist of polymerase chain reactions (PCR) and gel electrophoresis (Goodwin et al., 1995, Ward and Bateman, 1999, Fouly and Wilkinson, 2000, Rachdawong et al., 2002). However, difficulties remain in determining *Ggt* from related *Gaeumannomyces* spp. and *Phialophora* spp.

Molecular-based assays have also been devised to detect and quantify the amount of *Ggt* inoculum presence in soil and/or plant tissue (Ophel-Keller et al., 1995, Herdina and Ophel-Keller, 1996) and to subsequently predict and forecast the risk of take-all in wheat crops (Herdina and Roget, 2000, Ophel-Keller et al., 2008, Bithell et al., 2012a). The amount of initial *Ggt* inoculum in the soil is an important factor in the development of take-all disease. Ophel-Keller et al. (2008) developed a DNA-based assay to extract and quantify the amount of combined soil inoculum for *Ggt* and *Gga*, as well as *Gga* alone which allowed the amount of *Ggt* inoculum to be extrapolated. The *G. graminis* assay was commercialised in Australia as a DNA-based soil testing service, named Predicta B ® (B = broadacre) and provided by the South Australian Research and Development Institute (SARDI), along with the identification of additional soilborne pathogens: cereal cyst nematode (*Heterodera avenae*, *Rhizoctonia barepatch* (*Rhizoctonia solani AG8*), Fusarium crown rot (*Fusarium pseudograminearum* and *Fusarium culmorum*), root lesion nematode (*Pratylencgus neglectus* and *P. thornei*), stem nematode (*Ditkylenchus dipsaci*) and blackspot of peas (*Mycosphaerealla pinodes*, *Phoma medicaginis* var. *pinodella* and *Phoma koolunga*). The soil test predicts which of these pathogens pose a risk in the farmer's soil and therefore provides the farmers with advice for their cropping

programme and management options. The take-all qPCR prediction test has been evaluated in New Zealand, leading to commercial use of the test (Bithell et al., 2012a). Interestingly, Bithell et al. (2012b) found that compared to *Ggt*, *Gga* is uncommon in soil from wheat crops in New Zealand and the authors suggested that oats could be used as a break crop to manage take-all. This is also the case in the UK, *Gga* is generally confined to the west of the country. The DNA-based method has also been compared to the pot bioassay, as described above, and a positive relationship has been found between the amount of *Ggt* DNA and the percentage of roots infected in the pot bioassay (Gutteridge et al., 2008, Bithell et al., 2009). A concern with the use of DNA-based method over the pot bioassay assay is that the method could overestimate inoculum levels due to detecting DNA of dead mycelium, yet Herdina et al. (2004) found that the amount of *Ggt* DNA in dead mycelia declined by 99.8% in soil after 4 days of incubation and that it would play a small part in the total DNA level. A limitation of the DNA-based predictive test is that it does not detect *Ggt* directly. Lately, Keenan et al. (2015) have developed an alternative qPCR assay that can directly measure *Ggt* DNA from the wheat roots and can provide detection of *Ggt* DNA from both symptomatic and non-symptomatic root tissue. The specificity of the qPCR assay was confirmed by differentiating *Ggt* from *Gga* and *G. cylindrosporus*, as well as from additional soil-borne fungi and oomycetes including *Fusarium* spp., *Pythium irregulare* and *Rhizoctonia solani*.

### 1.4.5 Environmental factors favouring the development of take-all disease

Take-all infection develops best in soil that is well-structured and aerated, with a pH of between 5.5 and 8.5 and temperature in the range of 5°C to 30°C (Hornby et al., 1998). Take-all severity is largely dependent upon environmental conditions. Temperature and soil moisture conditions prior to the sowing of a susceptible crop influence the level of *Ggt* inoculum surviving saprotrophically in the soil (Macnish, 1973, Wong, 1984, Bithell et al., 2009) and affect the development and severity of take-all disease of subsequent crops (Hornby, 1981). *Gaeumannomyces graminis* var. *tritici* typically survives the intercrop period best during dry and cold conditions since the fungus is a poor saprotrophic competitor and these conditions inhibit

microbial activity, whereas warm and moist soils induce a decline in *Ggt* inoculum (Macnish, 1973, Wong, 1984, Bithell et al., 2009). Wong (1984) tested the survival of the take-all fungus and *Phialophora* fungi under different soil temperatures and soil moistures and found that survival was highest under cool and dry soil, followed by warm and dry soil.

Development of severe 'take-all years' is enhanced by warm winter conditions followed by a wet spring (Take-all in winter wheat-management guidelines, Autumn 2006, AHDB). However, above-ground symptoms can additionally be exacerbated by dry weather in the summer (Hornby et al., 1998). High rainfall in the spring and summer months, as well as high rainfall in the previous season, has been linked with severe 'take-all years' in Australia (Roget and Rovira, 1991, Bithell et al., 2013). Irrigation has been found to increase take-all severity during a dry season (Pillinger et al., 2005, Bithell et al., 2013). Yet *Ggt* growth is reduced if the soil becomes too saturated, thought to be due to the depleted oxygen levels in the soil (Heritage et al., 1989) or an indirect effect from the activities of antagonistic microbes (Grose et al., 1984).

## 1.5 Take-all control methods

### 1.5.1 Cultural control

The most effective form of cultural management is the use of crop rotations. The agricultural practice of a one-year break from a susceptible crop, results in the decline of *Ggt* inoculum levels and a reduction in the risk of disease development in subsequent crops. This is due to the poor saprotrophic survival of the fungus in the absence of a host crop and *Ggt* becomes uneven amongst the soil and begins to decompose (Hornby et al., 1998). Non hosts are typically broad-leaved crops but oats can also be used if *Gga* is absent. However, the removal of cereal volunteers in the break crop is important since the survival of these weakens the effectiveness of a break crop by carrying over take-all inoculum (Jenkyn et al., 1998). The same is true of many grass weed species, particular bromes and couch grass. An earlier sowing date of second wheat crops (Bithell et al., 2009) as well as an earlier sowing date of first wheats through to a fourth wheat (Smiley, 2009), have been shown to increase the risk of take-all infection. This allows for a shorter interval between

harvesting and sowing of the next susceptible crop, allowing less time for the breakdown of *Ggt* inoculum and exposing the crop to inoculum for a longer time period (Gutteridge and Hornby, 2003). A delayed sowing date can therefore be used to reduce the severity of take-all disease (Colbach et al., 1997, Gutteridge and Hornby, 2003, Smiley, 2009). However, this comes at a risk of reducing the yield potential, especially if there is a large amount of rainfall during October preventing the farmer being able to get onto their land to sow the crop, reducing the growing season.

Agricultural cultivation can also be important in reducing disease severity, although studies have shown mixed results. Ploughing can help to bury *Ggt* inoculum in the soil, bringing less infective soil to the top and reducing the potential of early root infection and increasing decomposition of the fungus (Take-all in winter wheat-management guidelines, Autumn 2006, AHDB). Ploughing can also help to control volunteers (Hornby et al., 1998) and weeds (Gutteridge et al., 2006). Direct drilling, the process of sowing the seed without prior cultivation, has had mixed effects on the severity of take-all disease. Rothrock (1987) found that direct drilling reduced the take-all incidence and severity, whereas Moore and Cook (1984) reported an increase in take-all incidence and severity from direct drilling when compared to ploughing.

Crop nutrition can play a major role in the development of plant diseases. Phosphate deficiency is thought to favour the development of take-all disease (Hornby et al., 1998). Gutteridge et al. (1996) found that the level of phosphate in the soil should be at least 20 mg/kg before a susceptible crop is sown, in order to reduce the risk of take-all. It is vital to correct phosphate deficiency prior to sowing the crop, since Gutteridge et al. (1996) found that take-all severity was not reduced if phosphate was added post-sowing. Potassium deficiency, and to a lesser extent manganese and sulphur deficiencies (Hornby et al., 1998) and copper and zinc deficiencies (Reis et al., 1982) should also be corrected prior to the sowing of a host crop.

### 1.5.2 Biological control

A number of microorganisms have been found to suppress take-all disease, including those involved in Take-all Decline (TAD) and *Phialophora* spp. belonging to the *Gaeumannomyces-Phialophora* complex. Additional microorganisms have also been found to provide a level of biocontrol against take-all, including arbuscular mycorrhiza fungi. Khaosaad et al. (2007) found a systemic bioprotectional effect of an arbuscular fungus, *Glomus mosseae*, to *Ggt* infection in barley. Furthermore, an endophytic fungus *Piriformospora indica,* isolated from India (Verma et al., 1998), has been found to colonise wheat roots and can inhibit the growth of *Ggt* (Ghahfarokhy et al., 2011). The fungus has also been found to increase resistance to a number of wheat pathogens including fusarium (Rabiey et al., 2015). Endophytic bacteria have also been found to provide a level of protection to take-all. Liu et al. (2009) isolated a *Bacillus subtilis* strain from wheat roots that was found to inhibit *Ggt* growth *in vitro* as well as reducing the level of take-all infection in wheat roots during field experiments. So far none of these have been commercialised.

#### 1.5.2.1 Take-all decline

Take-all Decline (TAD) is a phenomenon that results in the biological control of take-all. Experimental evidence for the phenomenon was first described in 1960s at Rothamsted Research and involves the natural soil suppression to take-all disease (Hornby et al., 1998). Take-all decline has been reported in the UK, Europe, the United States of America (USA), Australia and New Zealand. The development of TAD has been found to be similar across countries even though a range of soil types and climates exists (Raaijmakers and Weller, 1998). The underlying pattern of TAD development is consistent with the requirement of growing consecutive wheat crops, the presence of *Ggt* and severe take-all in previous wheat crops in the rotation (Raaijmakers and Weller, 1998, Weller et al., 2002). Field studies have shown that TAD could be initiated from as early as a third wheat crop, if the second wheat crop suffered from a severe 'take-all year' (Bateman and Hornby, 1999, Bithell et al., 2013).

Take-all decline is exploited by farmers, knowingly or not, through the agricultural practice of continuous cereals (Cook, 2003). Take-all decline however is not always

effective, especially if weather and soil conditions are favourable to the development of the disease (Hornby et al., 1998). It is currently unknown what the exact microbial species and suppressive processes that contribute to this natural phenomenon and studies have suggested that a different microbial composition is responsible for TAD in different geographical regions (de Souza et al., 2003). A number of studies have found that *Pseudomonas* spp. are involved in TAD throughout the world and have been found to be responsible for suppressiveness of the soil (Cook and Rovira, 1975, Weller et al., 1988, Raaijmakers and Weller, 1998, de Souza et al., 2003).

Fluorescent *Pseudomonas* spp., that produce the 2,4-diacetylphloroglucinol (2,4-DAPG) antibiotic, are a key component of the microbial community in TAD of soil in the USA and Netherlands (Raaijmakers and Weller, 1998, Weller et al., 2007). Raaijmakers and Weller (1998) found that take-all suppression was lost when the fluorescent *Pseudomonas* spp. were eliminated from the soil and gained with the addition of *Pseudomonas* strains into TAD soil. Cook et al. (2002) conducted field trials to determine the effect of seed treatments involving a *Bacillus* sp. and *Pseudomonas* spp., including the *Pseudomonas* strain from Raaijmakers and Weller (1998) study, on root diseases. Unfortunately, these seed treatments did not indicate a measurable reduction of take-all disease. Take-all decline is likely to be a complex community effect in many soils and does not solely rely on *Pseudomonas* spp. A range of microbial populations are now thought to be associated with the suppressiveness of TAD (Sanguin et al., 2009, Chng et al., 2015).

#### 1.5.2.2 *Phialophora* spp. within the *Gaeumannomyces-Phialophora* complex

*Phialophora* fungal species belonging to the genus *Gaeumannomyces*, colonise the roots and stems of cereals and a number of grasses (Holden, 1976) and form the *Gaeumannomyces-Phialophora* complex (Wong, 1981). *Phialophora graminicola* (Walker, 1980) (teleomorph *Gaeumannomyces cylindrosporus* (Hornby et al., 1977)) and *Phialophora* sp. lobed hyphopodia (Deacon, 1974) (teleomorph *Gaeumannomyces graminis* var. *graminis* (Walker, 1972)) were isolated from grass (Scott, 1970) and *Phialophora radicicola* (Cain, 1952) isolated from maize (*Zea mays*) (McKeen, 1952). Ward and Bateman (1999) found that ITS sequence analysis

indicated that *P. radicicola* was identical to *Gaeumannomyces graminis* var. *maydis*, a take-all fungus from China that infects maize (Yao et al., 1992), and virtually identical to *Phialophora zeicola*, a root pathogen that also infects maize, found in France and South Africa (Deacon and Scott, 1983). *Phialophora* sp. with lobed hyphopodia and *P. radicicola* are rarely pathogenic to cereals roots whereas *P. graminicola* is completely non-pathogenic (Holden, 1976). Fungal runner hyphae invade the outer cortical layers of the roots, but unlike the take-all fungus do not extensively colonise the stele or penetrate cortical cells (Holden, 1976, Bryan et al., 1995). The *Phialophora* spp. produce sub-epidermal vesicles, representing growth cessation structures (Deacon, 1974). The fungi therefore do not destroy vascular tissue and do not cause disease symptoms (Bryan et al., 1995).

*Phialophora graminicola* and *Phialophora* sp. lobed hyphopodia occur naturally in UK grasslands (Deacon, 1973b) and have been found to suppress take-all disease of wheat (Deacon, 1973a, Slope et al., 1979, Gutteridge et al., 2007). Yet *Phialophora* sp. lobed hyphopodia is thought to be less common in the UK (Hornby et al., 1998). The fungi inhabit the same ecological niche of *Ggt* (Wong, 1981) and can rapidly establish high population levels in grasslands and colonise successive cereal crops, though at deteriorating levels (Deacon, 1973a). Crop rotations involving ley grasses and clover can delay the onset of take-all disease by limiting the build-up of *Ggt* (Deacon, 1973a). Laboratory experiments involving grasses and cereals, artificially inoculated with *P. graminicola*, have indicated a reduction of take-all disease on wheat roots (Balis, 1970, Holden, 1974, Speakman et al., 1978a). Field trials have confirmed that prior infection of the root system by *P. graminicola,* after a grass-clover ley, has also been found to restrict the growth of take-all disease in subsequent wheat crops (Deacon, 1973a, Deacon, 1976, Slope et al., 1979). The degree of suppression is dependent upon the quantity of roots colonised by *P. graminicola* and the exposure time prior to contact with *Ggt* (Balis, 1970). Grasses have been found to selectively encourage *P. graminicola* over *Ggt* in naturally inoculated soils (Speakman et al., 1978b) and could be utilised as an opportunity for disease management. The use of grass species sown into field margins, along with the introduction of *Phialophora* spp. antagonists, could potentially act as a control

of take-all disease (Gutteridge et al., 2007). Bateman et al. (2005) however suggested that grass species, such as rye-grass, suppress take-all microbiota antagonists that are responsible for take-all decline. Currently, in the UK the use of grass leys in arable rotations is uncommon.

*Phialophora* spp. appear to displace the take-all fungus by prior colonisation (Wong, 1981). It has been suggested that *P. graminicola* and *Phialophora* sp. lobed hyphopodia restrict the development of *Ggt* in cereal and grass roots by increasing host resistance (Holden, 1974, Wong, 1975). Laboratory tests have indicated that the fungi do not directly suppress the hyphae of *Ggt* on agar media (Hornby, 1983) and antibiotics are not produced by the fungi (Wong, 1981). Speakman and Lewis (1978) found that *P. graminicola* and *Phialophora* sp. lobed hyphopodia caused widespread lignification of the stele in wheat roots. This is thought to restrict *Ggt* from dispersing longitudinally in the stele when both species colonise a root, limiting *Ggt* internal proliferation.

A number of field studies in Australia have applied artificial *Phialophora* inoculum to investigate the potential of reducing take-all disease in wheat roots in the field (Wong and Southwell, 1980, Wong et al., 1996). However, studies have found that a significantly greater ratio of *Phialophora* inoculum to *Ggt* inoculum is required in order to protect wheat roots. Wong et al. (1996) demonstrated that isolates of *Phialophora* sp. lobed hyphopodia gave effective control of take-all disease when artificial inocula was applied in Australian fields. Grain yields were increased 33-45% when 60 kg/ha of artificial oat grain inoculum of *Ggg* and *Phialophora* sp. lobed hyphopodia was applied to soil at the time of sowing and 21-44% when 30 kg/ha of the inoculum added. Wong and Southwell (1980) also found that a large amount of artificial *Phialophora* inoculum was needed to protect wheat roots, with a 3:1 ratio of *Ggg*:*Ggt*. However, economically significant protection against take-all was only demonstrated at sites with a low disease pressure from take-all. Mathre et al. (1998) also found that a large amount of artificial *Phialophora* inoculum, 54 kg/ha or more, was needed to provide a level of take-all control in wheat in Montana, USA.

All of the above studies used inocula that were uniformly dispersed throughout the experimental soil and this would not be the case in natural field conditions as inoculum would be generally lower and fragmented amongst the soil (Wong, 1981). The *Phialophora* spp. would also need to be tested in fields containing natural populations of take-all, since both studies used artificial *Ggt* inoculum. For viable field use artificial *Phialophora* spp. inoculum dose levels would need to be considerably reduced. However, it is unclear whether sufficient *Phialophora* populations would be able to establish from such a small amount of inoculum. The success of a biocontrol agent relies upon the rapid colonisation of wheat roots by the *Phialophora* spp. and persistence of the fungal species at a high inoculum level during and between cereal crop rotations (Wong, 1975, Wong, 1981). Take-all inoculum in the soil also needs to be at a low level.

Wong (1975) suggested that pelleting wheat seeds with the mycelium of *Ggg* could ensure that wheat roots became infected with the biocontrol agent before the roots come into contact with *Ggt*. Interestingly, *P. graminicola* cannot grow onto stems and this is seen as problematic in using the species as a seed inoculum as the fungus would not be able to grow along the subcrown internode of crown roots, preventing/limiting root-root contact (Deacon, 1974). Wong (1998) has patented a method and composition for the control of take-all disease of susceptible cereals and grasses through the composition of at least one *Phialophora* sp. lobed hyphopodia isolate which indicates cold tolerance. Wong et al. (1996) stated that Bio-Care Technology Pty Ltd., an Australian biotechnology company, were in the process of commercialising a *Ggg* and *Phialophora* sp. lobed hyphopodia isolate as biocontrol agents, as of yet no commercial use has been documented and pelleting wheat seeds with *Phialophora* spp. is not currently utilised as a method of biological control against take-all disease. Inconsistency in the performance of biocontrol agents often prevents extensive commercial use (Weller et al., 1988).

Gutteridge et al. (2007) has explored the potential of *Phialophora* spp. suppressing take-all disease in UK wheat fields. Natural populations of *Gaeumannomyces cylindrosporus* (anamorph: *P. graminicola*) were more effective at suppressing take-all disease compared to artificial inoculation of soil. The introduction of *Phialophora*

spp. in UK arable soils may have difficulties in establishing viable populations due to the complex biology of the soil and through competition with the natural microbiota (Hornby et al., 1998). Artificial inoculation of *Phialophora* spp. into UK arable fields is not currently feasible. Natural *Phialophora* populations do not build-up under cereals crops in the UK and for successful use in the field need to be introduced via grass leys (Deacon, 1973a) or through direct inoculation of the soil. The manipulation of natural populations of *Phialophora* spp. to provide protection against take-all is not utilised in the UK, due to the financial loss of incorporating grasses into cereal rotations (Hornby et al., 1998).

Combining microorganisms as a form of biocontrol for *Ggt* could replicate natural ecological processes in the soil associated with take-all suppression (Pierson and Weller, 1994, Duffy and Weller, 1995). Duffy and Weller (1995) looked at combining *Ggg* and fluorescent pseudomonas bacterial strains to soil in growth chambers and found that *Ggt* was suppressed. The combination however did not enhance *Ggt* suppression in field conditions compared to results for each single treatment.

### 1.5.3 Chemical control

There are currently two commercially available fungicides that are effective as seed treatments for controlling take-all, fluquinconazole (Dawson and Bateman, 2001) and silthiofam (Schoeny and Lucas, 1999). Fluquinconazole is commercially marketed as Jockey® and is a quinazoline-based triazole that inhibits sterol biosynthesis in fungal cell membranes and provides a broad spectrum of activity against a range of foliar and seed-borne diseases (Dawson and Bateman, 2000). Silthiofam is commercially available as the seed treatment Latitude®, specific to take-all and is thought to target the mitochondrial adenine nucleotide transporter (Joseph-Horne et al., 2000). Bateman et al. (2008) found that silthiofam was slightly more effective at reducing take-all infection and giving a higher yield potential than fluquinconazole. The authors suggested that this is because silthiofam is more effective at controlling primary infection of the roots (Bailey et al., 2005) whilst fluquinconazole is more effective at controlling secondary root-to-root infection. The 2016-2017 field season will be the last season that fluquinconazole will be

available since the chemistry has been withdrawn under the Plant Protection Products Regulation (EC) No 1107/2009.

Insensitivity to silthiofam has been found to occur naturally within *Ggt* field populations, without prior exposure to the fungicide (Carter et al., 2003, Freeman et al., 2005, Yun et al., 2012). Freeman et al. (2005) found that there was variable evidence for the selection of the fungicide for insensitive *Ggt* isolates. However, it has been reported that silthiofam efficacy to take-all has been declining in farmer's fields in China and a study by Yun et al. (2012), reported that the effectiveness of silthiofam for controlling take-all disease was low when wheat roots were infected with silthiofam-resistant *Ggt* isolates.

Additional fungicides for take-all control include the foliar strobilurin sprays azoxystrobin, commercially available as Amistar®, and fluoxastrobin, commercially available as Fandango®. It has been suggested that rain is required in order to allow these fungicides to penetrate the soil and therefore protect the root system, indicating that environmental conditions are important around the time of application as they influence the effectiveness of the fungicides (Gutteridge and Hammond-Kosack, 2008).

### 1.6 *Gaeumannomyces graminis* genome

The *Gaeumannomyces graminis* (var. *tritici* R3-111a-1) annotated genome was generated and released by the Broad Institute (www.broadinstitute.org) in April 2011 (Okagaki et al., 2015). The genome sequence, assembly and protein coding genes annotation of *Ggt* was published with a size of 43.62Mb and encoding 14,463 protein-coding genes (Okagaki et al., 2015). The *Ggt* genome was sequenced to 25X coverage. Sequencing of the *Ggt* genome is part of the *Magnaporthe* comparative project, a collaboration between the International Rice Blast Genome Consortium and the Broad Institute, that includes sequencing and providing access to fungal genomes from the Magnaporthaceae family. The genome of *Magnaporthe oryzae* (strain 70-15) (Dean et al., 2005, Okagaki et al., 2015), the rice blast fungus, and the draft assembly of *Magnaporthe poae* (strain ATCC 64411) genome (Okagaki et al.,

2015), the causal agent of summer patch disease in turf grasses, have also been published as part of this study.

A reliable transformation system of *Ggt* is currently lacking and an absence of available mutants makes it extremely difficult to study gene function within the species. Published transformation studies have shown a low transformation rate and variable stability within and between strains (Pilgeram and Henson, 1990). The study by Bowyer et al. (1995) is currently the only successful example of targeted gene disruption in *Gaeumannomyces graminis*. The authors produced avenacinase mutants of *Gga* that were no longer able to infect oat roots. The development of an effective transformation system would facilitate the genetic analysis of the biology and infection process of the take-all fungus.

## 1.7 Use of take-all resistant germplasm, species and varieties

### 1.7.1 Oats

*Gaeumannomyces graminis* var. *tritici* is known to grow epiphytically over the cortex of oat roots (Huber and McCaybuis, 1993) but cannot infect the roots due to the presence of the antifungal inhibitor avenacin (Turner, 1960, Crombie et al., 1986). The presence of runner hyphae therefore does not indicate *Ggt* root infection (Huber and McCaybuis, 1993). Turner (1940) stated that oats were considered a valuable control measure against *Ggt* if a wheat crop was sown following oats, generating a yield similar to a crop after fallow. Unlike their resistance to *Ggt*, oats are susceptible to *Gga* infection. *Gaeumannomyces graminis* var. *avenae* isolates produce avenacinase, an enzyme that deglucosylates the avenacin compound into a less toxic form and allows infection (Turner, 1961, Osbourn et al., 1991). *Fusarium avenaceum* and *P. graminicola* are also able to detoxify the plant compound (Crombie et al., 1986).

Avenacin is a plant secondary metabolite comprising four structurally related molecules: avenacin A-1, A-2, B-1 and B-2. Avenacin A-1 is the most common extract found in young oat roots, around 70% of the total avenacin content, and the most fungitoxic (Crombie and Crombie, 1986). The A-1 compound provides a barrier to soil-borne pathogens since the metabolite is localised to the epidermal

cell layer of roots (Osbourn et al., 1994). Avenacin production is limited to species within the *Avena* genus and is therefore absent in wheat (Osbourn, 2003). This suggests that introgression of avenacin biosynthesis into wheat could prove extremely difficult. *Avena longiglumis*, a diploid oat species, has been shown to lack the production of the avenacin A-1 and is therefore susceptible to *Ggt* infection (Osbourn et al., 1994). Yet *A. longiglumis* cannot easily hybridise with other diploid oat species. Avenacin-mutants for the diploid species *Avena strigosa* have been produced and are being studied to determine the functional activity and biosynthetic pathway of avenacin (Papadopoulou et al., 1999, Wegel et al., 2009). It has been suggested that even if wheat could be genetically engineered to produce avenacin, isolates insensitive to avenacin or with the ability to detoxify the metabolite are likely to be selected (Cook, 2003).

### 1.7.2 Rye and triticale

Rye (*Secale cereale*) is highly resistant to take-all and the wheat x rye hybrid cereal triticale has been shown to comprise an intermediate level of resistance between wheat and rye (Nilsson, 1969, Scott, 1981, Rothrock, 1988, Bithell et al., 2011, McMillan et al., 2014). Rye has been found to exhibit disease escape through the production of new roots, as well as demonstrating tissue-based resistance (Skou, 1975). The genetic basis of resistance in rye is currently unknown. Difficulties exist when attempting to assess the genetic basis of resistance due to a lack of variation between rye varieties in their level of susceptibility to take-all (McMillan et al., 2014).

Wilkes et al. (1999) suggested that take-all resistance in rye could be the result of plant extracts inhibiting the fungus. The authors found that both rye and triticale contain 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) in their roots, whilst wheat roots only DIMBOA. *Gaeumannomyces graminis* var. *tritici* growth was inhibited when the combination of both DIMBOA and DIBOA were added to the *Ggt* nutrient media (Wilkes et al., 1999).

### 1.7.3 Wheat

Strong, consistent and repeatable resistance to take-all disease is lacking in wheat (Scott, 1981) and differences between wheat varieties in their level of resistance have been generally considered too small to be of beneficial use (Hornby et al., 1998). Studies have been conducted in Australia that have identified a level of resistance in wheat screened under natural populations of take-all in the field. Penrose (1991) found that there were differences between wheat varieties in the percentage of infected roots across two field sites. Further studies have shown a greater difference in susceptibility to take-all between two wheat varieties, Temu89-72 and Bayonet (Penrose, 1992, Penrose, 1995). Penrose (1995) carried out five field trials at different sites in Australia and found that Temu89-72 was consistently more resistant to natural populations of the take-all fungus than Bayonet. More recent work based at Rothamsted Research (RRes), within the Wheat Genetic Improvement Network (WGIN) and the Wheat Improvement Strategic Programme (WISP), has identified a number of hexaploid wheat varieties that have shown moderate resistance to take-all disease in the field (see Chapter 3). The most resistant hexaploid varieties will be further characterised in third wheat field trials within this PhD project.

Nilsson (1969) found that accessions of the diploid wheat *Triticum monococcum*, varied considerably in their susceptibility to take-all with a number of accessions exhibiting a degree of resistance to the disease. Scott (1981) reviewed the literature on host plant resistance to take-all and suggested that moderate resistance to take-all is likely to be more frequent in diploid wheat species than polyploid species and resistance comparable to rye has not been reported. More recently, McMillan et al. (2014) have reported of *T. monococcum* accessions that have consistently illustrated moderate to high levels of resistance to take-all across different field seasons and under different disease pressures at RRes. The most resistant *T. monococcum* accessions were intermediate between rye and triticale in their level of susceptibility (McMillan et al., 2014). The genetic basis of resistance in the *T. monococcum* accessions is unknown and will be explored within this PhD project (see Chapter 4). Additionally, the diploid goat grass (*A. tauschii*) is also currently

being screened for susceptibility to take-all in field trials at RRes, under the WISP programme, to identify resistance to take-all that could potentially be introgressed into modern elite varieties (Vanessa McMillan, personal communication). Barley is slightly less susceptible to take-all than wheat (Hornby et al., 1998), yet there has been little evidence of consistent differences in the level of take-all susceptibility in barley cultivars (Nilsson, 1969).

## 1.8 Project aims and objectives

The overall aim of the PhD project was to characterise a range of diploid and hexaploid wheat germplasm that possess a promising level of take-all resistance under field conditions. In addition, the susceptibility of hexaploid wheat germplasm to populations of *Phialophora* species under field conditions and pot bioassays was to be explored.

**Objective 1:** To further characterise the most take-all resistant hexaploid wheat lines from the Watkins and Gediflux collections under field conditions including assessing both above and below ground phenotypes and testing the use of *in situ* continuous soil moisture probes to evaluate upper root function of a selected subset of take-all susceptible and resistant varieties (Chapter 3).

Hypothesis: Soil surrounding the most heavily take-all diseased plants will be the wettest.

**Objective 2:** To characterise the genetic basis and complexity of take-all resistance in the *Triticum monococcum* MDR037 (Susceptible) x MDR046 (Resistant) mapping population under field conditions (Chapter 4).

Hypothesis: The genetic basis of take-all resistance in *T. monococcum* will be controlled by multiple QTLs.

**Objective 3:** To gather a *Phialophora* isolate collection to enable the genomes of three UK *Phialophora* species to be sequenced and to identify regions of uniqueness in each of the three *Phialophora* genomes in order to design a species-specific diagnostic assay in the future (Chapter 5).

Hypothesis: The three *Phialophora* draft genomes will be similar with a limited but useable level of polymorphism.

**Objective 4:** To explore the susceptibility of current UK AHDB Recommended List (RL) winter wheat varieties and other cereal species to *Phialophora* fungi under both field and controlled environment conditions (Chapter 6).

Hypothesis: Susceptibility to *Phialophora* will vary across the RL winter wheat varieties.

# Chapter 2. General materials and methods

## 2.1 *Phialophora* isolations from root tissue

In order to develop a *Phialophora* isolate collection, *Phialophora* spp. were isolated from root tissue of baited soil cores, sampled from fields across the Rothamsted Farm, in Chapter 5. *Phialophora* spp. were also isolated from a subset of soil core bioassay roots from the field trial in Chapter 6. *Phialophora* colonised root pieces were cut (~1 cm long segments) and surface sterilised for 5-10 min in sodium hypochlorite (1:5 dilution with sterile distilled water), triple rinsed in sterile distilled water and blotted dry on sterilised filter paper. Potato dextrose agar (PDA) (Sigma Aldrich®, Dorset, UK) (see media recipe below) amended with penicillin (50 µg per PDA plate) and streptomycin (50 µg per PDA plate) was poured into Petri dishes (90 mm diameter x 15 mm height) in a sterile air flow hood and surface sterilised root pieces were plated onto the PDA. Plates were incubated at 15°C for ~ two weeks. Cultures resembling *Phialophora* were transferred using a sterile tooth pick onto fresh PDA amended with penicillin (50 µg per PDA plate) and streptomycin (50 µg per PDA plate) and incubated at 15°C for ~ two weeks. This method was repeated if the plates continued to be contaminated with other bacterial or fungal cultures. Uncontaminated *Phialophora* cultures were then transferred onto fresh PDA without antibiotics. Plates were incubated at 15°C until plates were confluent (~ two weeks). Plates were then stored at 4°C for future use.

**Media recipe**

**Potato dextrose agar (PDA):** 39 g dissolved in 1 litre of $H_2O$. Autoclaved to sterilise.

### 2.1.1 Maintenance and long term storage of *Phialophora* isolates from the field

*Phialophora* field isolates were stored on PDA plates at 4°C for experimental use throughout the year. Two 1 $cm^2$ agar plugs were cut from a confluent PDA plate for each isolate and placed in a sterile universal bottle with 15 ml of sterile distilled water for long-term storage (Boesewinkel, 1976). Two bottles were set up for each isolate. The bottles were stored at room temperature.

### 2.1.2 DNA extraction and PCR amplification for fungal species identification

A 1cm³ agar plug was cut from the stored *Phialophora* PDA plates and transferred to a fresh PDA plate, amended with penicillin (50 µg) and streptomycin (50 µg), in a sterile air flow hood. The plates were incubated at 15°C until confluent, ~ two weeks. Mycelium was then scraped using a sterile toothpick from the confluent cultures on PDA plates for each of the isolates in a sterile flow hood (Fraaije et al., 1999). Mycelium was freeze-dried during the day and overnight (~24 hours) and then ground using a 3 mm steel ball bearing in a FastPrep at 4 m/s for 20 secs. The water bath was preheated to 65°C. DNA was extracted using prepared DNA extraction buffer (see solution recipes below). In a fume cupboard, β-mercaptoethanol was added to the DNA extraction buffer (for a total volume of 0.1%) before adding 600 µl of the DNA extraction buffer to each sample. Samples were vortexed and incubated in the water bath at 65°C for 30 mins before adding 300 µl of cold (-20°C) 7.5 M ammonium acetate (see solution recipes below). Samples were incubated on ice for 30 mins before centrifugation at 11,000 *g* for 10 mins. The supernatant was transferred into fresh Eppendorf tubes containing an equal volume of cold (-20°C) isopropanol. Samples were then incubated at -20°C for 2 hours before centrifugation at 11,000 *g* for 10 mins. The supernatant was discarded and the pellet was washed twice in cold (-20°C) 70% ethanol. Samples were inverted 15 times and centrifuged at 11,000 *g* for 3 mins. Ethanol was discarded and pellets were air-dried for 30 mins before being resuspended in 100-400 µl distilled $H_2O$. Extracted DNA was quantified using the Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA) and stored at -20°C for future use.

**Solution recipes**

**Ammonium Acetate (7.5 M):** 28.905 g ammonium acetate in 50 ml sterile distilled $H_2O$ (filter sterilise). Store at -20°C.

**Sodium Dodecyl Sulfate (SDS) (2%):** 10 g in 500 ml sterile distilled $H_2O$. Heat to dissolve.

**T.E.N. Buffer (2X):** Make up to 450 ml and adjust pH to 8.0 before increasing to 500ml.

| | |
|---|---|
| NaCl | 14.61 g |
| Tris. HCl | 31.52 g |
| EDTA | 11.9 g |
| Distilled $H_2O$ | 500 ml |

**DNA Extraction Buffer:** Make up solution in a pre-sterilised 500 ml bottle.

| | |
|---|---|
| T.E.N. (2x) | 190ml |
| SDS (2%) | 190ml |
| Phenanthroline monohydrate (5 mM) | 0.36g |
| Polyvinylpyrrolidone (2%) | 8.0g |
| | 400ml |

PCR was then performed using a G-STORM GS1 thermal cycler (G-STORM, Somerton, Somerset, UK) to amplify internal transcribed spacers (ITS)5F (GGAAGTAAAAGTCGTAACAAGG) and ITS4R (TCCTCCGCTTATTGATATGC) regions. A 20 µl reaction was set up and contained 10 µl REDTaq® Ready Mix™ (Sigma Aldrich®, Dorset, UK), 1 µl of each primer (10 µM) (synthesised by Sigma Aldrich®, Dorset, UK), 6 µl of sterile distilled $H_2O$ and 2 µl of template DNA (100 ng/µl) or 2 µl of sterile distilled $H_2O$ for the negative control. Amplification conditions consisted of an initialisation step at 95°C for 5 min followed by 30 cycles with a denaturation step at 95°C for 30 secs, an annealing step at 55°C for 1 min, an extension step at 72°C for 1 min, and a final extension step at 72°C for 10 min and final hold at 4°C. PCR products (4 µl) were separated by a 1.2% agarose gel electrophoresis in 1 x TBE (see solution recipe below) with a 100 bp DNA ladder (Thermo Fisher Scientific, USA), stained with ethidium bromide (Sigma Aldrich®, Dorset, UK) and ran at 80 V for 40 mins before visualising under UV light at a wavelength of 302 nm in a Gene Genius Bioimaging System (Syngene, Cambridge, UK). PCR products (16 µl) were purified using a Qiagen QIAquick PCR Purification Kit as per the manufacturer's

instructions (Qiagen Ltd., Manchester, UK). The PCR products were quantified using the Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA).

Sequences were sent off to Eurofins MWG Operon (Ebersberg, Germany) for sequencing analysis. The National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) was then used to enter returned sequences into the nucleotide Basic Local Alignment Search Tool (BLAST) database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to find regions of similarity between the sequences for species identification.

**Solution recipe**

**1 x TBE buffer:** Dissolve 10.8 g Tris and 5.5 g Boric acid in 900 ml distilled $H_2O$. Add 4 ml 0.5 M EDTA (pH 8.0). Increase volume to 1 litre. Store at room temperature.

### 2.2 Field trials

Field trial designs were generated by statistician Rodger White using CycDesigN (VSN International Ltd, Hemel Hempstead, UK) and a randomised block design was calculated for each trial. Field trials were established on the Rothamsted Farm, on flinty clay soil of the Batcombe soil series, in a first or third wheat situation. First wheat field trials were carried out after a one year break crop of oilseed rape (*Brassica napus*), in order to screen the build-up of *Phialophora* and the susceptibility of current elite wheat varieties to *Phialophora* colonisation (2015/R/WW/1516 and 2016/R/WW/1620 Chapter 6) (Figure 2.2). Cereal germplasm was screened for take-all resistance in a third wheat situation, after two consecutive winter wheat crops, to try and ensure a high natural take-all disease pressure (2014/R/WW/1419 and 2015/R/WW/1510 Chapter 3 and 2014/R/WW/1418 and 2015/R/WW/1512 Chapter 4) (Figure 2.2). Fertilisers, pesticides and growth regulators were applied according to standard Rothamsted farm practice with the exceptions of no take-all seed treatments or fungicides active against take-all as well as no growth regulators applied to the diploid *Triticum monococcum* wheat plots as well as only one dose of herbicides (Pacifica®) being applied to the herbicide sensitive diploid wheat plots.

**Key:**

2014/R/WW/**1419**: Hexaploid wheat possessing take-all resistance 2013-2014 trial (Chapter 3)

2015/R/WW/**1510**: Hexaploid wheat possessing take-all resistance 2014-2015 trial (Chapter 3)

2014/R/WW/**1418**: *Triticum monococcum* MDR037 (S) X MDR046 (R) 2013-2014 trial (Chapter 4)

2015/R/WW/**1512**: *Triticum monococcum* MDR037 (S) X MDR046 (R) 2014-2015 trial (Chapter 4)

2015/R/WW/**1516**: Susceptibility of elite wheat to *Phialophora* spp. 2015 trial (Chapter 6)

2016/R/WW/**1620***: Susceptibility of elite wheat to *Phialophora* spp. 2016 trial (Chapter 6)

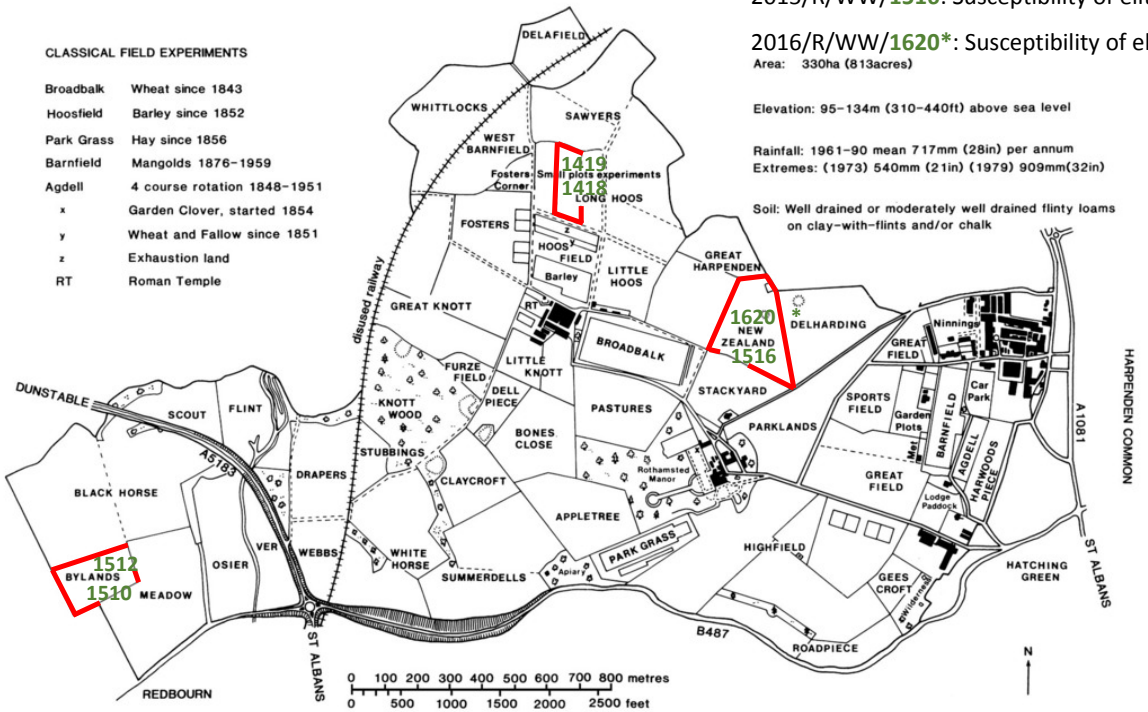



Figure 2.1. Rothamsted Farm map with the highlighted field sites and field trial codes (year/R/WW/code) for the six field trials conducted within this PhD project. * Field trial not assessed within the timeframe of the PhD project.

A number of above-ground phenotypic crop measurements were recorded for each field trial. Ear emergence/days to anthesis and leaf senescence were recorded by visually evaluating the middle of each plot, avoiding the edge effect of the small plots, and assessing plants that represented the majority of the plot. Ear emergence was recorded up to the date of anthesis, growth stage 61, using the Zadoks Decimal Code for the growth stages of cereals key (Zadoks et al., 1974). Flag leaf senescence was recorded visually during the afternoon. A senescence key, obtained from Simon Orford at the John Innes Centre, was used to score each plot using a scale of 0-10, 0 representing no senescence and 10 representing fully senesced flag leaves (Figure 2.2). Plots were scored when they reached days to full leaf senescence, score 10 on the scale. Plant height was measured from the base of the plant to the top of the awn for two plants in the middle of each plot and an average was calculated.

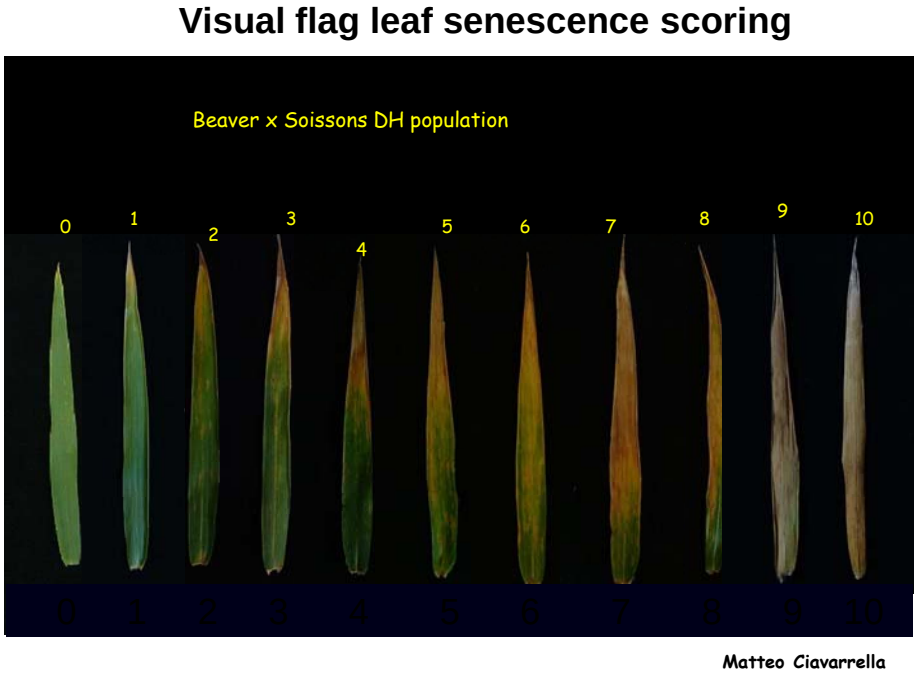



Figure 2.2. Visual flag leaf senescence scoring system. Received from Simon Orford at the John Innes Centre.

### 2.2.1 Seed preparation

The third wheat field trial screening of the most resistant Watkins and Gediflux varieties (Chapter 3) was hand harvested from a field trial in 2012 to provide seed for the 2014 field trial (2014/R/WW/1419). The 2014 field trial was then hand harvested to provide seed for the 2015 field trial (2015/R/WW/1510) (Figure 2.2). The ears were threshed by hand and a seed cleaning machine (designed and built by the Facilities Department at RRes) was used to separate the seed from the chaff. The seed for the third wheat field trial screening the diploid *T. monococcum* mapping population (Chapter 4), was bulked up in the glasshouse and threshed by hand. The seed for the first wheat field trial, screening the susceptibility of current elite wheat varieties to *Phialophora* fungi in Chapter 6, was provided by the relevant breeding companies and was supplied pre-threshed.

Germination tests were then carried out for all of the cereal germplasm prior to seed preparation for each individual field trial, with the exception of the seed for the diploid *T. monococcum* mapping population field trials that was grown under glasshouse conditions since the seed quality was expected to be high and limited seed was available. One hundred seeds were germinated for each genotype on damp tissue for around 7 days. The number of germinated seeds were recorded and a percentage of germination calculated. If germination rates were extremely poor, then the germination was tested for additional seed stocks and/or the seed rate was increased.

Thousand grain weights (average of two counts) were then calculated for all of the field trial cereal germplasm, except for the diploid *T. monococcum* mapping population seed. The weight for 350 seeds was then calculated and weighed out for each variety, representing a seed rate of 350 per $m^2$ plot (6 rows x 1 m length). The seed rate was increased for the varieties that had a poor germination rate and no availability of additional seed stocks. The *T. monococcum* seed was counted by hand for a seed rate of 80 seeds per 4 row x 0.6 m length plot. The seed was then loaded into cassettes and then onto trays for each field trial. Trays were labelled with the field trial number and the field trial plan row number. One tray was used per row in the field, the only exception was the *T. monococcum* field trials where

one tray was used per row for block 1 and block 2 of the field trial design, along with one tray for block 3 and block 4 and one tray for block 5. Seed was distributed between four or six vertical wells, corresponding to the four or six rows in each individual plot in the field, of each cassette. The cassettes were then loaded onto the trays. String (white polypropylene twine) was tied around the trays and loaded cassettes to prevent the cassettes sliding off during transport to the farm. The string was then removed and each tray was loaded into the Haldrup SB-25 Belt Cone plot drill (Haldrup) when the trial was drilled by Rothamsted Farm staff.

### 2.2.2 Plant sampling and storage of take-all disease and *Phialophora* colonisation field trials

Plant samples were taken during each summer, from the medium milk development stage onwards, to assess for take-all root infection (Chapter 3 and Chapter 4) and for *Phialophora* colonisation (Chapter 6). String (white polypropylene twine) was labelled with the field trial code and plot number. The plot number was also written on the ring of the label to prevent losing the sample if the label was damaged or removed whilst processing the sample. Plant samples were taken from three 20 cm lengths of row per plot, separate rows were sampled at different positions. Each of the three plant samples were tied onto the string. Plant samples were transferred back to the field laboratory and were washed free of soil. The plant samples were cut to just above the stem bases and then placed into the polytunnel to air dry (Figure 2.3). Samples were turned after 2-3 days and removed after approximately 5 days, when fully dried. The dried plant root systems were then stored at room temperature in the field laboratory and assessed for either take-all disease or *Phialophora* colonisation throughout the year.



Figure 2.3. Photograph of whole adult plant root systems drying in the polytunnel outside of the field laboratory at RRes.

### 2.2.3 Take-all disease assessments

The dried plant root systems were soaked in water for approximately 20 minutes. Any remaining soil was then washed off and the root systems were immersed in water in a white dish. The roots were scored for take-all infection in order to calculate a take-all index (TAI) (Bateman et al., 2004). The proportion of roots infected were visually assessed and graded in the categories of no infection, slight 1 (1-10% roots infected), slight 2 (11-25%), moderate 1 (26-50%), moderate 2 (51-75%) and severe (75-100%). A take-all index was then calculated: (1 x percentage plants slight 1) + (2 x percentage plants slight 2) + (3 x percentage plants moderate 1) + (4 x percentage plants moderate 2) + (5 x percentage plants severe); divided by the number of categories (5); maximum TAI 100.

## 2.3 Microscopy

A LEICA M205 FA stereomicroscope and associated LAS-AF6000 software (Leica Microsystems Ltd, UK) were used for microscopic visualisation and image capture, from the attached digital camera, of take-all root infections and *Phialophora* root colonisations. Seedling roots were submersed in water in a Petri dish and visualised under the stereomicroscope. Scale bars were generated by the LAS-AF6000 software.

## 2.4 Photography

A Sony A390 digital SLR camera was used for image capture throughout the thesis. An iPhone 5C phone was also used for image capture of some of the field trial photographs. Aerial images of the field trials were captured by the Visual Communications Unit at RRes. A Nikon D3x digital SLR camera, fitted with a 60 mm macro lens, was used by RRes photographer Graham Shephard to capture images of the field sampled whole adult root systems.

## 2.5 Statistics

Statistical analyses were performed in the software Genstat (VSN International Ltd, Hemel Hempstead, UK). The percentage take-all disease data and *Phialophora* colonisation data were transformed using the logit transformation and were statistically analysed by Rodger White throughout the chapters. The above-ground crop measurements for the each of the field trials were statistically analysed by Rodger White throughout the chapters. Within the analyses, auto-regressive models were used for spatial adjustment of the field trials to account for the degree of patchiness of take-all in both the y axis and the x axis across the trial site if required. It is likely that plots closer together could be more strongly correlated than plots further apart due to the formation of take-all patches in the field. The change in deviance was checked for all analyses to support fitting auto-regressive models for spatial adjustment or whether a less complex model should be used. The *P* value threshold was set at ≤0.05 for all statistical tests. Specific statistical analyses are detailed in individual chapters.

# Chapter 3. Field evaluation of the upper root function of susceptible and moderately resistant hexaploid wheat varieties in a high take-all risk 3rd wheat situation

## 3.1 Introduction

The Watkins Landrace Wheat Collection and the cultivars from the European Union project on Genetic Diversity in Agriculture: Temporal Flux (Gediflux), along with a number of elite wheat varieties, have previously been screened at Rothamsted Research (RRes) for resistance against take-all disease in the field (http://www.wheatisp.org/). Moderate resistance has been identified in a number of varieties (Vanessa McMillan, personal communication). One of the main aims of this PhD project was to further characterise the 25 most take-all resistant varieties and to test the upper root function of selected varieties through the use of *in situ* soil moisture sensors. Replicated field trials over two seasons were carried out in a third wheat situation with additional control varieties. Soil moisture sensors were inserted into select plots of resistant varieties as well as susceptible control varieties to phenotype root function throughout the growing season in a high take-all risk situation.

Within the core research project of the Department for Environment, Food and Rural Affairs (Defra) funded Wheat Genetic Improvement Network (WGIN) programme (http:\\www.WGIN.org.uk) two unexplored wheat germplasm collections, The Watkins Landrace Wheat Collection and the Gediflux collection, have been resurrected at the John Innes Centre by single seed decent (SSD). The Watkins collection consists of landrace cultivars of bread wheat from 32 countries that were collected by A. E. Watkins in the late 1920s-1930s (Miller et al., 2001). The collection provides a glimpse of the genetic diversity of the landrace cultivars prior to the start of modern breeding (Wingen et al., 2014) and originally contained over 7000 accessions (Miller et al., 2001). The majority of these accessions were lost during the Second World War and today the collection consists of 826 land race accessions collected from across Europe, Asia and Africa (Wingen et al., 2014). In

contrast, the Gediflux project was set-up to explore the impact that intensive breeding had on the genetic diversity of European crops, specifically barley, maize, potato and wheat (Reeves et al., 2004). The Gediflux winter wheat collection contains 281 semi-modern elite cultivars of European winter wheat that have occupied at least 5% of winter wheat acreage in a North European country during 1945-2000 along with 229 varieties from the UK National List in commerce in the late 1990s (Reeves et al., 2004).

The Watkins collection and the Gediflux collection provide the opportunity of allele discovery for important traits that could be used in modern wheat genetic improvements. The Watkins collection has already been used to locate new alleles or genes for resistance against leaf rust (*Puccinia triticina*) (Dyck, 1994) and stripe rust (*Puccinia striiformis*) (Bansal et al., 2011). Within the WGIN and the Wheat Improvement Strategic Programme (WISP), the Watkins collection and the Gediflux collection are being screened to identify useful resistance traits against plant pathogens and pests and Burt et al. (2014) have identified a single gene for resistance against eyespot (*Oculimacula yallundae* and *Oculimacula acuformis*).

As part of the WGIN and WISP programmes, ~800 Watkins lines and Gediflux varieties have previously been screened in the field at RRes for susceptibility to take-all disease over a five-year period (Figure 3.1). In the initial screening, within the WGIN project, a single replicate of each variety was tested. In 2010, the most susceptible varieties were discarded from the field trial and a single replicate of each variety was again tested. A significant number of susceptible varieties were then discarded after the take-all assessment of the 2010 trial. This allowed a replicated field trial, within the WISP project, during the field seasons 2011 of 5 replicates of 156 Watkins lines and Gediflux varieties. Take-all is renowned to be a patchy disease in the field and replication of varieties is essential to assess for susceptibility to take-all disease. Field screening of the Watkins and Gediflux collections have shown that a number of varieties indicate moderate resistance to take-all infection. During 2012, a high disease pressure year, the 156 Watkins lines and Gediflux varieties from the 2011 trial were again evaluated in the field, along with a number of elite wheat varieties, were screened with five replicates per

variety along with the three control cereal varieties of rye (highly resistant), triticale (moderately resistant) and winter wheat variety Hereward (highly susceptible) (Figure 3.1). Field results indicated a range of susceptibility to take-all disease with a subset of lines revealing a similar take-all index score to the moderately resistant control of triticale. Due to the high take-all disease pressure seen in 2012, 25 of the most resistant wheat varieties from the 2012 field trial were chosen to take forward for further field characterisation and evaluation of upper root function in this PhD project.

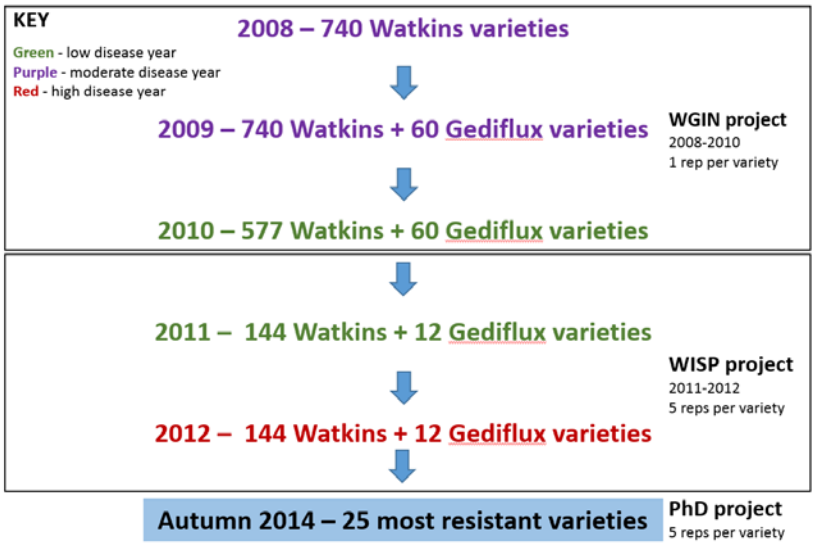



Figure 3.1. Number of varieties from the Watkins collection and Gediflux collection screened in the field across 5 years at Rothamsted Research. (McMillan & Gutteridge, unpublished data, WGIN & WISP).

Phenotyping for root traits in the field can be extremely difficult. Techniques are often laborious and require the destruction of field plots during sampling (Paez-Garcia et al., 2015). Laboratory-based methods can be used to look at root traits of seedlings and young plants (Paez-Garcia et al., 2015) but results must translate into traits found in adult root systems in the field to be useful for future breeding programmes. Results from controlled environment studies, especially when examining a single factor, should be taken with caution as the root architecture may vary once the plant material is transferred to the field due to the increased range of parameters encountered by the roots and the greater number of

environmental changes occurring (Rich and Watt, 2013). It is currently not possible to track in a dynamic way root architecture throughout the season in the field. An alternative is to excavate plants throughout the season to determine the function of the root systems during specific stages of plant development, however this method is timely and cannot be undertaken in small field trial plots. Excavating adult plants post-anthesis, allows the root systems of mature plants to be evaluated (Trachsel et al., 2011, Bucksch et al., 2014). The wheat varieties studied in this chapter were sampled by excavating adult plants during the summer to allow the roots to be examined for take-all infection. This allowed the root systems in the top 15-25 cm to be evaluated and a number of phenotypic root trait measurements were recorded, consisting of number of seminal roots, number of crown roots and the total root dry weight of the sample root system. Previous studies have shown that take-all infected rye plants can produce extra crown root as a form of disease escape (Skou, 1975). This trait has not been reported in a *Triticum* species. Measurements were taken to examine the different root traits in the presence of take-all disease and to determine whether there was a relationship between individual root traits and the level of take-all disease.

Another alternative to excavating plants to determine root functionality is to measure the soil drying profile. Traditional methods of determining soil moisture were often calculated by taking a soil core, drying the sample to 105°C before weighing the sample and dividing the dried sample by the sample volume (Blake and Hartge, 1986). This would provide a soil bulk density measurement, the ratio of the mass of the dried sample to the bulk volume of the soil. The water content of the sample is calculated by taking the mass of the dry soil away from the mass of the wet soil and then dividing this by the mass of the dry soil (Gardner et al., 1991). This method is time consuming and problems arise when sampling stony soil (Gardner et al., 1991). From the 1950s onwards, portable neutron probes were used in the field to measure soil water content (Stone et al., 1955, Evett and Steiner, 1995). The neutron probe method utilises hydrogen in its ability to slow down fast neutrons from a radioactive source (Gardner et al., 1991). The radioactive source and the neutron detector are within the probe. Before the

neutron probe can measure the soil water content, access tubes must be inserted into the field, which allows the neutron probe to be used at a range of depths (Gardner et al., 1991). The majority of hydrogen present in the soil is found as water molecules. As the water content of the soil increases, the number of fast neutrons that are slowed down increases (Gardner et al., 1991). However, due to the radioactive sources of the neutron probes, their use poses a potential health hazard. Dielectric soil moisture sensors are seen as an attractive alternative to the radioactive neutron probes (Huang et al., 2004). Dielectric soil moisture sensors measure the volumetric soil water content by responding to changes in the dielectric constant of the soil (Gardner et al., 1991). The dielectric sensors can be buried *in situ*, at differing depths, allowing the water content of the soil profile to be measured throughout the season. The plant available soil moisture can then be quantified to infer root activity of the plant.

It is known that plant disease pressure can be affected by the level of soil moisture and previous studies have explored using soil moisture for plant disease forecasting (Bom and Boland, 2000, Matheron and Porchas, 2005, Palmieri et al., 2006). Take-all is known to be more severe during years of high rainfall and therefore under high soil moisture levels (Hornby et al., 1998, Cook, 2003, Bithell et al., 2013). Take-all infection is known to disrupt the uptake of water and nutrients to the plant. Pillinger et al. (2005) carried out two field trials, sown with the winter wheat variety Equinox, to establish whether the disruption of water uptake was from reduced root growth or the reduction in the efficiency of the root system. The study found that the take-all epidemics did not begin to increase until late May, whereas typically the rate of root growth increases from early April (Gregory et al., 1978). Therefore, during these late epidemics, take-all reduced the ability of the wheat to uptake water from colonising and reducing the functionality of the root system and not from preventing root growth (Pillinger et al., 2005).

One of the main aims of this PhD was to determine whether there was a relationship between the upper root function of the varieties and their take-all index score. The working hypothesis was that soil surrounding the most heavily take-all diseased plants would be the wettest. By inserting soil moisture sensors

into the plots of both take-all susceptible and resistant varieties, the soil drying of the plots could be measured throughout the field season and therefore explore whether soil moisture sensors can differentiate between a healthy roots system and a take-all disease root system. It may be that some varieties are still able to maintain upper root function despite a high level of take-all disease and the soil moisture sensors would therefore indicate an increased drying effect within the plot. The study aimed to determine whether soil moisture sensors could be used as a non-destructive discriminatory tool for field screening of varieties for take-all infection.

This PhD study reports on two field trial seasons characterising the 25 most resistant hexaploid wheat varieties to take-all, along with a number of additional diploid and hexaploid wheat varieties and control cereal varieties. The varieties were further characterised via above-ground and below-ground phenotypic crop measurements as well as measuring root functionality through the use of soil moisture sensors. Above-ground phenotypic crop measurements consisted of recording plant height, days to anthesis and days to leaf senescence. The below-ground phenotypic crop measurements were carried out post-plant sampling and involved tiller counts, seminal and crown root counts from a subsample of the plant samples as well as carrying out total root dry weights of the sampled whole adult root systems. *In situ* soil moisture sensors were inserted into the plots for five varieties, three selected from the most resistant varieties and two selected from the susceptible control varieties. The soil moisture sensors monitored the soil water content throughout the field season to determine whether the susceptible lines to take-all disease had a higher level of soil moisture surrounding the roots due to loss of function of the roots to uptake water to the plant.

## 3.2 Materials and methods

### 3.2.1 Characterising the 25 most take-all resistant hexaploid lines in the field

Two field trials were carried out to screen the 25 most promising resistant lines to take-all from the Watkins wheat collection, the Gediflux wheat collection and elite hexaploid wheat varieties. The field trials were all established on the Rothamsted Farm (Table 3.1) and were carried out in a third wheat situation, to try to ensure a high natural take-all disease pressure. A randomised block design was calculated for each trial by statistician Rodger White (see Appendix 3.1 for field plans). Seed was prepared for both field trials as detailed in Chapter 2.

In the 2013-2014 field season, five replicates of the 25 most take-all resistant hexaploid lines were screened in the field along with 5 replicates of the additional lines of the moderately resistant *Triticum monococcum* accessions MDR031 and MDR046 and the susceptible *T. monococcum* accessions MDR037 and MDR043. These diploid wheat accessions were included to provide a comparison between the most resistant hexaploid wheats and the most resistant diploid wheats used in Chapter 4. Control lines consisted of five replicates of rye (highly resistant to *Gaeumannomyces graminis* var. *tritici* (*Ggt*)), triticale (moderately resistant to *Ggt*) and spring wheat variety Paragon (susceptible to *Ggt*) and 40 replicates of the winter wheat variety Hereward (highly susceptible to *Ggt*) (Table 3.2). Lines were hand-harvested from the field trial to provide seed for the 2014-2015 replicated field trial (Table 3.3) as detailed in Chapter 2.

Table 3.1. Experimental field trial design for the 2013-2014 (2014/R/WW/1419) and 2014-2015 (2015/R/WW/1510) field trials.

| Field trial | Rothamsted field and soil type | Previous crop and variety | Treatments | Plot size | Seed rate per plot | Sowing date |
|---|---|---|---|---|---|---|
| **2014/R/WW/1419** | Long Hoos 5<br>Batcombe - Carstens with sandier inclusions | Winter wheat<br>Conqueror | 18 Watkins lines, 3 Gediflux lines, 4 hexaploid varieties, 4 *Triticum monococcum* accessions, 4 controls | 4 rows x 1m length | 350 | 31/10/2013 |
| **2015/R/WW/1510** | Bylands<br>Typical Batcombe | Winter wheat<br>Conqueror | 18 Watkins lines, 3 Gediflux lines, 4 hexaploid varieties, 4 *Triticum monococcum* accessions, 4 controls, 4 additional varieties | 4 rows x 1m length | 350 | 24/10/2014 |

Dielectric soil moisture sensors (ML2x-ThetaProbe Soil Moisture Sensor, Delta-T Devices Ltd., Cambridge, UK) were inserted in four replicates of five varieties to evaluate the functioning of the upper root systems of both take-all resistant and susceptible varieties. The three moderately take-all resistant varieties chosen were the winter wheat varieties Duxford and Hereford and the Watkins collection line 777. The two take-all susceptible varieties chosen were firstly the winter wheat variety Hereward, which is the highly susceptible take-all control variety and secondly the spring wheat variety Paragon, which is susceptible to take-all and is the parent of the Paragon x Watkins line 777 mapping population. In the 2013-2014 field trial the soil moisture sensors were split across four blocks, and then connected to a data logger in one of two heavy duty lockable boxes that were placed to the north and south of the trials (see Appendix 3.1). One of the Hereford replicates was not sown in the field trial due to a problem when drilling the trial, it was therefore decided that a soil moisture sensor would still be inserted into this plot and that it would be used as a fallow control (see Appendix 3.1 for field trial plans). Prior to inserting the soil moisture sensors into the field, the sensors were tested in the laboratory to ensure that the devices were consistent in recording soil moisture over time. The sensors were placed into damp sand before briefly being placed into a bucket of water and then into dry sand before being placed back into the damp sand to test that the sensors were consistent in measuring the level of drying in the damp sand. The sensors were then ready to insert into the field trial. Once plants had emerged, a depth of 15 cm was drilled in the centre of each plot at a 45° angle using a soil auger. The soil moisture sensor cable was threaded down a pole and this was used to insert and push the sensor into the hole at the depth of 15cm until the four stainless steel rods were set firmly into the soil (Figure 3.2). The hole was then re-packed with the excavated soil; care was taken to avoid over-packing the soil. The soil moisture sensors were placed at a 45° angle in order for running water, from rain events, to run down the side of the sensor and to be carried away from the four stainless steel rods of the sensor. Cables (between 5 m and 20 m lengths) were connected to the soil moisture sensors which ran parallel to the field plots and were securely fastened to the soil surface with steel pegs. The cables were connected to a data logger (DL2e, Delta-T Devices Ltd., Cambridge, UK)

in the corresponding box. Data loggers were programmed using Ls2WIN 1.0 SR10 software (Delta-T Devices Ltd., Cambridge, UK) and set to record every hour. The data loggers were connected to a 12V leisure battery as an external power supply. A separate sensor (SM300, Delta-T Devices Ltd., Cambridge, UK) was inserted into one of the Hereward plots, roughly in the middle of the field trial, and set to record the soil temperature. The sensor was again placed at a depth of 15 cm at a 45° angle and connected to a data logger (GP1, Delta-T Devices Ltd., Cambridge, UK) that was set to record every hour and the data logger was left on the surface of the soil next to the plot. Data from all sensors was downloaded onto a laptop at roughly fortnightly intervals. Soil and temperature sensors were left to record until 11$^{th}$ July 2014 (Table 3.3) and removed after plant sampling.

In 2014-2015, a replicated field trial was sown with 5 replicates of additional lines of the spring wheat variety Frument, a sister variety to the variety Hereford, as well as South African spring wheat lines SA1, SA2 and SA3 that are thought to have some level of resistance to take-all in the field. These additional varieties are part of the larger field trial and will not be analysed within the PhD project due to time constraints. Thirty replicates were sown of the winter wheat control variety Hereward (highly susceptible to *Ggt*) (Table 3.2). To ensure a balanced block design of the 2014-2015 field trial with the additional lines, a number of 'dummy' plots were included by statistician Rodger White. These 30 'dummy' plots were also sown with the field trial control variety Hereward to enable a greater number of plots to be included in the statistical analysis, a total of 60 Hereward plots.

Soil moisture sensors were again inserted into four replicates of the five varieties (Duxford, Hereford, Watkins line 777, Hereward and Paragon) as described for the 2013-2014 field trial. The four replicates were however split across five blocks of the field trial (see Appendix 3.1 for field trial plan). Two additional soil moisture sensors were inserted into the bare soil to the north and south of the field trial as two fallow controls (Appendix 3.1). A separate sensor (SM300, Delta-T Devices Ltd., Cambridge, UK) was again inserted into one of the Hereward plots, roughly in the middle of the field trial, and set to record the soil temperature as described for

2014 field trial. Soil and temperature sensors were left to record until the 10th August 2015 (Table 3.3) and were removed after plant sampling.

Sampling methods and measurements taken over the two field seasons are detailed in Table 3.3. Field trial plots were weeded by hand on 18/06/15. Above-ground phenotypic crop measurements were recorded and adult plant samples were taken in July for each plot, as described in Chapter 2. The whole trial was assessed for take-all infection in the 2013-2014 field trial, whereas only the field plots with the inserted soil moisture sensors were assessed for take-all infection in the 2014-2015 field trial due to time constraints. Subsamples of nine plants per plot were chosen at random to perform tiller counts, seminal root counts and crown root counts for all 33 varieties in the 2013-2014 field trial and for just the 20 plots with the inserted soil moisture sensors in the 2014-2015 field trial (Table 3.2). Total root dry weights of the whole sampled adult root systems were carried out for the 19 plots with inserted soil moisture sensors in the 2013-2014 field trial and the 20 plots with inserted soil moisture sensors in the 2014-2015 field trial. The plant samples were dried in the oven at 40 °C once the take-all assessments and root counts had been performed. The whole root systems were then cut from all plants within the plant samples, dried in the oven at 80 °C for 48 hours and then weighed to provide a total root dry weight.

Table 3.2. Cereal genotypes screened for resistance to take-all over the two field seasons of 2013-2014 and 2014-2015.

| Variety/line | Wheat collection | Country of origin | Year of collection/first listed | Treatment in field trials | Field season screened |
|---|---|---|---|---|---|
| G16 M. Tempo | Gediflux | Federal Republic of Germany | 1940-50 | 25 most take-all resistant | 2014 & 2015 |
| G40 Marco | Gediflux | Belgium | N/A | 25 most take-all resistant | 2014 & 2015 |
| G51 Kontrast | Gediflux | Germany | 1990 | 25 most take-all resistant | 2014 & 2015 |
| 20 | Watkins | India | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 114 | Watkins | Yugoslavia | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 151 | Watkins | Portugal | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 156 | Watkins | Yugoslavia | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 168 | Watkins | China | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 197 | Watkins | India | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 202 | Watkins | Spain | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 204 | Watkins | India | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 487 | Watkins | USSR | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 488 | Watkins | USSR | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 489 | Watkins | USSR | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 492 | Watkins | Spain | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 500 | Watkins | Iraq | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 739 | Watkins | India | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 777[1] | Watkins | Finland | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 776 | Watkins | USSR | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 794 | Watkins | USSR | 1930 | 25 most take-all resistant | 2014 & 2015 |
| 821 | Watkins | China | 1930 | 25 most take-all resistant | 2014 & 2015 |

Table 3.2. Continued.

| Variety/line | Wheat collection | Country of origin | Year of collection/first listed | Treatment in field trials | Field season screened |
|---|---|---|---|---|---|
| Cassius | Elite | UK | 2009 | 25 most take-all resistant | 2014 & 2015 |
| Duxford[1] | Elite | UK | 2008 | 25 most take-all resistant | 2014 & 2015 |
| Hereford[1] | Elite | Denmark | NR 2007[3] | 25 most take-all resistant | 2014 & 2015 |
| Solstice | Elite | UK | 2002 | 25 most take-all resistant | 2014 & 2015 |
| Rye (var. Carotop) | Elite | UK | 2006 | Control | 2014 & 2015 |
| Tritcale (var. Trilogie) | Elite | UK | 2008 | Control | 2014 & 2015 |
| Hereward[1] | Elite | UK | 1991 | Control | 2014 & 2015 |
| Paragon[1] | Elite | UK | 1999 | Control | 2014 & 2015 |
| Frument[2] | Elite | Denmark | 2000s | Additional | 2015 |
| SA1[2] | Elite | South Africa | 1995 | Additional | 2015 |
| SA2[2] | Elite | South Africa | | Additional | 2015 |
| SA3[2] | Elite | South Africa | | Additional | 2015 |
| MDR031 (R) | *Triticum monococcum* | Turkey | 1927 | Additional | 2014 & 2015 |
| MDR043 (S) | *Triticum monococcum* | Greece | 1950 | Additional | 2014 & 2015 |
| MDR046 (R) | *Triticum monococcum* | Romania | 1970 | Additional | 2014 & 2015 |
| MDR037 (S) | *Triticum monococcum* | Armenia | 1934 | Additional | 2014 & 2015 |

[1] Varieties had soil moisture sensors inserted into four plots of the 2013-2014 and 2014-2015 field trials.

[2] Additional varieties in the 2014-2015 field trial were not analysed within this PhD project.

[3] NR = Not recommended (first candidate year).

Table 3.3. Sampling and measurement details throughout the two field trial seasons.

| Field trial | Sampling method | Units per plot | Date measured/sampled | Growth stage (GS) |
|---|---|---|---|---|
| **2014/R/WW/1419** | Ear emergence | GS | 27/05/14 - 20/06/14 | 39-61 |
| | Leaf senescence | 0-10 | 26/06/14 - 29/07/14 | 73-92 |
| | Plant heights | cm | 24/06/2014 | 69-75 |
| | Plant samples | 3 x 20cm | 15 - 16/07/14 | 91 |
| | Soil moisture | $m^3/m^3$ | 22/11/13 - 11/07/14 | 11-91 |
| | Hand harvest | | 24 - 25/07/14 | 92 |
| **2015/R/WW/1510** | Ear emergence | GS | 12/05/15 - 12/06/15 | 61 |
| | Leaf senescence | 0-10 | 09 - 27/07/15 | 77-87 |
| | Plant heights | cm | 08 - 09/07/15 | 77 |
| | Plant samples | 3 x 20cm | 09 - 10/07/15 | 77 |
| | Soil moisture | $m^3/m^3$ | 03/12/14 - 10/08/15 | 11-91 |
| | Hand harvest | | 05 - 06/08/15 | 91 |



Figure 3.2. Method of inserting soil moisture sensors into the 2013-2014 (2014/R/WW/1419) field trial.

The TAI and above-ground phenotypic crop measurements field trial data were analysed by statistician Rodger White. The take-all index (TAI) data was logit transformed before performing an Analysis of Variance (ANOVA) analysis on the disease data for the 2013-2014 field trial. The above-ground phenotypic crop measurements of plant height and ear emergence were statistically analysed through ANOVA and leaf senescence was statistically analysed through a Residual Maximum Likelihood (REML). The residual diagnostic plots were examined on the below-ground root measurements and a square root transformation was performed on the data for the number of seminal roots and number of crown roots as well as data for the number of tillers before carrying out an ANOVA analysis on the data. The total root dry weights were measured for the 19 plots of the five varieties Hereward, Paragon, Watkins line 777, Duxford and Hereford with *in situ* soil moisture sensors. Four replicates (four plots) of each of the wheat varieties had inserted soil moisture sensors with the exception of only three replicates (three plots) for the variety Hereford. This led to an unbalanced experimental design and therefore a REML analysis was carried out to analyse the total root dry weight data of the plant samples for the five varieties.

The experimental design of the collected 2014-2015 trial data was unbalanced due to the 20 plots, representing four field plots with *in situ* soil moisture sensors for five varieties, being split across five blocks and as a result each treatment was not equally repeated within the five blocks of the experiment. Therefore, the field trial data for the TAI, ear emergence, leaf senescence, plant height, number of seminal roots, number of crown roots, number of tillers and total dry weights of the whole plant samples were analysed using REML. Transformation of the below-ground root measurements of number of seminal roots, number of crown roots as well as the data for the number of tillers was not needed for the 2014-2015 data.

Ear emergence was analysed as days to anthesis from 1$^{st}$ May and the flag leaf senescence was analysed as days to leaf senescence from 1$^{st}$ June for both field trials. Spearman's rank correlation was used to test the association between the different above-ground phenotypic crop measurements and between the above-ground phenotypic crop measurements and the TAI for both field trials.

### 3.2.2 Soil moisture analyses

The dielectric soil moisture sensors (ML2x-ThetaProbe Soil Moisture Sensor, Delta-T Devices Ltd., Cambridge, UK) mV data output was converted to volumetric soil water content using the polynomial equation conversion in Figure 3.3. The volumetric soil moisture content is defined as the ratio between the volume of water present and the total volume of the soil (units are $m^3/m^3$) throughout the chapter (ThetaProbe Soil Moisture Sensor Type ML2x User Manual). The value of 0 $m^3$ water/ $m^3$ soil represents completely dry soil, whereas the value of 1 $m^3$ water/ $m^3$ soil represents pure water (ThetaProbe Soil Moisture Sensor Type ML2x User Manual). The polynomial relationship was used to enable accuracy over the full range of values, particularly when the soil became very wet in the organic soil with the soil moisture content measuring > 0.5 $m^3$ water/$m^3$ soil (ThetaProbe Soil Moisture Sensor Type ML2x User Manual).

$$\theta = \frac{\left[1.07 + 6.4V - 6.4V^2 + 4.7V^3\right] - a_0}{a_1}$$

Figure 3.3. Equation used to convert the ML2x-ThetaProbe Soil Moisture Sensor mV data to volumetric soil water content.

A daily average soil water content was then calculated for each day for each sensor. There was a large variability in the soil water content between the replicates of the individual soil moisture sensors for each variety, especially for the 2013-2014 field trial. This was probably related to differences in soil density. To compare soil water status between the different treatments the degree of saturation was used. This was estimated by the ratio of any given water content with the water content of the saturated soil. The daily rainfall and the potential soil moisture deficit (PSMD) were downloaded from the electronic Rothamsted Archive (e-RA) for both field seasons. The degree of saturation for each plot of each variety were plotted against the TAI of the plot for selected dates throughout the months of April, May, June and July for both years as well as for August for 2015 data. If possible, graphs were plotted on the same scale for the two field trials in order to compare differences between the two field season years.

A number of issues arose with the soil moisture sensors throughout the two field trial seasons. Connecting cables and soil moisture sensors were damaged in both seasons, leading to the failure of the sensors in their measurement of the soil water content. Cables and sensors were therefore replaced when necessary. During 2015, there was a problem with hares (*Lepus europaeus*) chewing through the cables and sensors of the plots located on the right-hand side of the field trial. An electric fence was erected, by farm staff, around the entire field trial site to help deter the hares from entering as well as covering the connecting cables on the right-hand side of the field trial with garden hose pipe (Homebase, UK) for added protection from the hares (see Appendix 3.1).



Figure 3.4. Photograph of the 2014-2015 (2015/R/WW/1510) field trial. An inserted soil moisture sensor can be seen in the bottom left plot and one of the two heavy durable lockable boxes housing the leisure battery and data logger can be seen at the far end of the trial. The electrical fence, to deter hares, can also be seen around the edge of the field trial. Photograph taken on 15/04/15.

## 3.3 Results

### 3.3.1 Performance of the cereal varieties to take-all disease in the field

#### 3.3.1.1 Weather conditions across the two field seasons

The monthly weather data for the two field seasons was downloaded from the Rothamsted electronic archive (e-RA) along with the departure from the 30 year means (1981-2010) (Table 3.4). The maximum and minimum mean temperatures for the 2013-2014 and 2014-2015 field trial seasons were similar to the 30 year means. There was a significant amount of rainfall in November 2015, the month after sowing the field trial, which was not seen in November 2014. The 2013-2014 season saw a milder winter and a warmer summer than the 2014-2015 season. The monthly rainfall for March, April, May and June were similar across the two years.

#### 3.3.1.2 Take-all susceptibility of the 2013-2014 field trial experiment

The 2013-2014 field trial screened 33 cereal varieties and a significant difference for the TAI was found across the varieties ($P$ = <0.001) (Table 3.5). The TAI for the control varieties ranged from 6.86 - 53.88. The TAI across the highly susceptible control variety Hereward was 53.88, indicating that a moderate level of disease was present across the field trial. The TAI across the 25 most take-all resistant wheat varieties ranged from 16.16 - 59.61, with the Watkins lines having a much larger range than the Gediflux varieties and the elite varieties. Of the five varieties with *in situ* soil moisture sensors, there was little difference in susceptibility between the take-all susceptible control variety Paragon and two of the three moderately resistant varieties, Watkins line 777 and Duxford, although a level of resistance was found for Hereford, relative to the TAI of the highly susceptible control variety Hereward. A subset of Watkins lines also indicated a level of resistance to take-all, namely 487, 500 and 739 (Table 3.5). There was little differentiation in the TAI of the parental accessions of the *T. monococcum* MDR037 (S) X MDR046 (R) mapping population. The *T. monococcum* parental accession MDR031, of the MDR043 (S) X MDR031 (R) mapping population, appeared to hold a level of resistance compared to the susceptible parental accession MDR043.

Table 3.4. Monthly weather summaries during 2013-2014 (2014/R/WW/1419) and 2014-2015 (2015/R/WW/1510) field trial seasons downloaded from e-RA.

| Monthly weather summary (Departure from the 30 year means (1981-2010) in brackets) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean temperature (°C) | | | | | |
| | | | Maximum | | Minimum | | Rainfall | |
| **Field season** | **Year** | **Month** | **(°C)** | **()** | **(°C)** | **()** | **Total (mm)** | **()** |
| **2013-2014** | 2013 | October | 15.3 | (+1.21) | 9.3 | (+2.15) | 108.8 | (+27.07) |
| **2014/R/WW/1419** | 2013 | November | 8.9 | (-0.85) | 3.6 | (-0.24) | 59.5 | (-17.13) |
| | 2013 | December | 9.1 | (+2.22) | 2.6 | (+0.92) | 123.1 | (+53.60) |
| | 2014 | January | 8.5 | (+1.83) | 2.9 | (+1.66) | 176.2 | (+106.25) |
| | 2014 | February | 9.0 | (+2.07) | 3.2 | (+2.31) | 141.9 | (+91.80) |
| | 2014 | March | 12.7 | (+2.84) | 3.4 | (+0.73) | 28.2 | (-22.57) |
| | 2014 | April | 14.8 | (+2.19) | 6.1 | (+2.12) | 31.5 | (-23.54) |
| | 2014 | May | 16.5 | (+0.41) | 8.3 | (+1.39) | 82.8 | (+28.14) |
| | 2014 | June | 20.1 | (+1.01) | 10.6 | (+0.88) | 30.5 | (-22.77) |
| | 2014 | July | 23.9 | (+2.11) | 13.3 | (+1.40) | 36.9 | (-13.00) |
| **2014-2015** | 2014 | October | 16.0 | (+1.97) | 9.8 | (+2.63) | 95.7 | (+14.05) |
| **2015/R/WW/1510** | 2014 | November | 11.3 | (+1.53) | 5.9 | (+2.06) | 108.2 | (+31.57) |
| | 2014 | December | 8.1 | (+1.20) | 2.1 | (+0.41) | 64.0 | (-5.57) |
| | 2015 | January | 7.5 | (+0.76) | 0.9 | (-0.32) | 81.9 | (+11.93) |
| | 2015 | February | 6.7 | (-0.21) | 0.8 | (-0.17) | 54.6 | (+4.42) |
| | 2015 | March | 10.2 | (+0.27) | 2.9 | (+0.27) | 26.1 | (-24.67) |
| | 2015 | April | 14.5 | (+1.85) | 4.0 | (-0.04) | 31.0 | (-24.05) |
| | 2015 | May | 15.5 | (-0.53) | 7.0 | (+0.12) | 68.4 | (+13.75) |
| | 2015 | June | 19.5 | (+0.33) | 9.2 | (-0.58) | 26.7 | (-26.55) |
| | 2015 | July | 21.4 | (-0.40) | 11.7 | (-0.15) | 132.6 | (+82.71) |
| | 2015 | August | 20.5 | (-1.10) | 12.1 | (+0.29) | 83.2 | (+19.49) |

#### 3.3.1.3 Take-all susceptibility of the 2014-2015 field trial experiment

The 2014-2015 field trial screened 37 cereal varieties for take-all susceptibility. However, due to time contraints only the five wheat varieties with *in situ* soil moisture sensors in the four field plots were assessed for take-all infection (Table 3.5). Although no significant difference for the TAI was found across the five varieties ($P$ = 0.077), a trend for a higher TAI was found for the two susceptible control varieties Hereward (TAI = 21.99) and Paragon (TAI = 11.51) and a lower TAI for the three varieties from the top 25 most resistant wheat varieites of Duxford (TAI = 1.20), Watkins line 777 (TAI = 2.77) and Hereford (TAI = 5.57) (Table 3.5). The TAI across the highly susceptible control variety Hereward (TAI = 21.99), indicated that a low level of disease was present across the field trial. During the 2014 field trial, there was less difference between the two susceptible varieties and the three resistant varieties. The ranking order of the TAI was not consistent amongst the five varieties across the two field trial seasons ($r_s$ = 0.100, $P$ = 0.196, n = 5).

Table 3.5. Susceptibility of cereal varieties to take-all disease in the 2013-2014 (2014/R/WW/1419) and 2014-2015 (2015/R/WW/1510) field trial seasons.

| | **Logit of take-all index (0-100), (back transformed means)** | | | |
|---|---|---|---|---|
| **Treatment** | **2014** | | **2015** | |
| **Controls** | | | | |
| Rye | -1.266 | (6.86) | | |
| Triticale | -0.650 | (20.91) | | |
| Hereward | 0.088 | (53.88) | -0.619[1] | (21.99)[1] |
| Paragon | -0.171 | (41.03) | -0.996[1] | (11.51)[1] |
| **Varieties** | | | | |
| G16 M. Tempo | -0.286 | (35.60) | | |
| G40 Marco | -0.303 | (34.78) | | |
| G51 Kontrast | -0.093 | (44.87) | | |
| 20 | 0.205 | (59.61) | | |
| 114 | -0.134 | (42.85) | | |
| 151 | -0.051 | (46.97) | | |
| 156 | -0.333 | (33.45) | | |
| 168 | -0.013 | (48.86) | | |
| 197 | -0.070 | (46.01) | | |
| 202 | -0.203 | (39.49) | | |
| 204 | 0.161 | (57.48) | | |

Table 3.5. Continued.

| | Logit of take-all index (0-100), (back transformed means) | | | |
|---|---|---|---|---|
| **Treatment** | **2014** | | **2015** | |
| **Varieties** | | | | |
| 487 | -0.462 | (27.90) | | |
| 488 | -0.334 | (33.38) | | |
| 489 | -0.345 | (32.88) | | |
| 492 | 0.048 | (51.92) | | |
| 500 | -0.789 | (16.60) | | |
| 739 | -0.805 | (16.16) | | |
| 776 | -0.342 | (33.05) | | |
| 777 | -0.163 | (41.44) | -1.693[1] | (2.77)[1] |
| 794 | -0.390 | (30.93) | | |
| 821 | -0.331 | (33.51) | | |
| Cassius | -0.022 | (48.43) | | |
| Duxford | -0.088 | (45.11) | -2.029[1] | (1.20)[1] |
| Hereford | -0.417 | (29.80) | -1.370[1] | (5.57)[1] |
| Solstice | -0.388 | (31.00) | | |
| MDR031 (R) | -0.574 | (23.61) | | |
| MDR043 (S) | -0.196 | (39.80) | | |
| MDR046 (R) | -0.247 | (37.41) | | |
| MDR037 (S) | -0.172 | (40.97) | | |
| d.f. | 32 | | 4 | |
| SED | 0.252 | | 0.470 | |
| F statistic | 3.65 | | 2.80 | |
| F probabilty | <0.001 | | 0.077 | |
| Grand mean | -0.277 | (37.17) | -1.341 | (8.61) |

[1] Five varieties with *in situ* soil moisture sensors represent only four plot replicates during 2014-2015 field trial.

#### 3.3.1.4 Above-ground phenotypic crop measurements of the 2013-2014 field trial experiment

The above-ground phenotypic crop measurements for the 2013-2014 field trial (2014/R/WW/1419) indicated that there were significant effects of cereal varieties on days to anthesis ($P$ = <0.001), days to leaf senescence ($P$ = <0.001) and plant height ($P$ = <0.001) (Table 3.6). Days to anthesis (from 01/05/14) ranged from 28 - 50 days across all genotypes (Table 3.6). The elite wheat varieties had a small range of 37.96-39 days, whereas the varieties from the Watkins and Gediflux collections had a large range of 11 days across the 21 varieties (32-43 days) and began to reach

anthesis at an earlier date than the elite varieties (Table 3.6). The *T. monococcum* accessions reached anthesis at the latest date. Days to leaf senescence (from 01/05/14) ranged from 40.68 - 53.96 (Table 3.6). The Watkins collection lines had a large range of 11 days to senescence (40.68 - 51.81 days) (Table 3.6). The three Gediflux varieties had a smaller range of only 3 days and senesced later than all but one of the Watkins lines (Table 3.6). The *T. monococcum* accessions senesced at a similar date to the Watkins lines. The elite varieties had a similar range and date of senescence as the Gediflux varieties (Table 3.6). Plant height ranged from 78.7 cm-148.4 cm across the genotypes (Table 3.6). As expected, the elite varieties indicated the shortest plant heights. The Watkins and Gediflux varieties had a higher range of 92.4 cm-132.4 cm, with two of the Gediflux varieties having a height at the bottom of the range (Table 3.6). The consecutive Watkins lines 487, 488 and 489 all had similar days to anthesis, days to leaf senescence and plant height (Table 3.6). These three cultivars are from the same source country, USSR, and have the same species name (*Triticum aestivum* var. *erythrospermum* (Korn.)).

The above-ground phenotypic crop measurements were then correlated against one another. A weak positive correlation was found between days to anthesis and days to leaf sensescence ($r_s$ = 0.275, *P* = 0.034, n = 33) (Table 3.7). A moderately negative correlation was found between plant height and leaf senescence ($r_s$ = -0.428, *P* = 0.003, n = 33). The taller the plant, the earlier the leaves senesced. The above-ground phenotypic crop measurements were then correlated against the TAI but no significant correlations were found (Table 3.7).

#### 3.3.1.5 Above-ground phenotypic crop measurements of the 2014-2015 field trial experiment

For the 2014-2015 field trial (2015/R/WW/1510), the above-ground phenotypic crop measurements were analysed only for the five vareties with *in situ* soil moisture sensors in the four field trial plots. The measurements indicated that there were signficant effects of cereal varieties on days to anthesis (*P* = <0.001), days to leaf senescence (*P* = 0.002) and plant height (*P* = <0.001) (). There was a trend for the varieties to reach anthesis at a later date in the 2014-2015 field season than during 2013-2014. The ranking order of the days to anthesis were

consistent across the 2013-2014 field trial and 2014-2015 field trial amongst the five varieties ($r_s$ = 0.825, *P* = 0.021, n = 5). There was a trend for the five varieties to reach leaf senescence at an ealier date than in 2013-2014. A large amount of variation was found across the two field trial seasons in the ranking order of days to senescence amongst the five varieties ($r_s$ = 0.100, *P* = 0.196, n = 5). There was a trend for a slightly shorter plant height in 2014-2015 compared to the plant height in the 2013-2014 field trial data. The ranking order of the recorded plant height was however found to be consistent across the two years amongst the five varieties ($r_s$ = 0.900, *P* = 0.004, n = 5).

The above-ground phenotypic crop measurements were then correlated against one another. A strong negative correlation was found between plant height and leaf senescence ($r_s$ = -0.700, *P* = 0.033, n = 5). The above-ground phenotypic crop measurements were then correlated against the TAI but there were no significant correlations found (Table 3.7).

Table 3.6. Above-ground phenotypic crop measurements of days to anthesis, days to leaf senescence and plant height of cereal varieties in the 2013-2014 (2014/R/WW/1419) and 2014-2015 (2015/R/WW/1510) field trials.

| Treatment | Days to anthesis from 1st May | | Days to leaf senescence from 1st June | | Plant height (cm) | |
|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| **Controls** | | | | | | |
| Rye | 28.00 | | 50.87 | | 147.35 | |
| Triticale | 28.00 | | 45.72 | | 116.65 | |
| Hereward | 39.00 | 42.00[1] | 51.55 | 50.08[1] | 82.84 | 80.15[1] |
| Paragon | 39.00 | 43.00[1] | 51.56 | 48.43[1] | 106.60 | 108.18[1] |
| **Lines** | | | | | | |
| G16 M. Tempo | 43.00 | | 53.96 | | 129.95 | |
| G40 Marco | 39.00 | | 53.57 | | 97.00 | |
| G51 Kontrast | 32.00 | | 50.82 | | 92.40 | |
| 20 | 38.20 | | 44.63 | | 128.80 | |
| 114 | 39.00 | | 45.20 | | 142.30 | |
| 151 | 33.40 | | 47.58 | | 119.45 | |
| 156 | 35.80 | | 45.93 | | 127.25 | |
| 168 | 33.20 | | 40.68 | | 122.25 | |

Table 3.6. Continued.

| Treatment | Days to anthesis Date from 1st May | | Days to leaf senescence Date from 1st June | | Plant height (cm) | |
|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| **Lines** | | | | | | |
| 197 | 42.20 | | 50.60 | | 131.85 | |
| 202 | 42.20 | | 51.81 | | 131.45 | |
| 204 | 41.40 | | 45.61 | | 148.40 | |
| 487 | 38.20 | | 43.88 | | 123.60 | |
| 488 | 38.20 | | 43.45 | | 123.70 | |
| 489 | 39.00 | | 47.29 | | 120.20 | |
| 492 | 38.20 | | 42.98 | | 129.05 | |
| 500 | 32.00 | | 44.84 | | 108.55 | |
| 739 | 39.00 | | 46.31 | | 132.35 | |
| 776 | 32.00 | | 44.80 | | 126.20 | |
| 777 | 32.00 | 39.00[1] | 44.57 | 42.99[1] | 131.90 | 130.16[1] |
| 794 | 39.00 | | 41.79 | | 132.20 | |
| 821 | 38.20 | | 42.85 | | 131.25 | |
| Cassius | 39.00 | | 53.19 | | 78.65 | |
| Duxford | 39.00 | 46.00[1] | 51.27 | 50.83[1] | 86.75 | 84.75[1] |
| Hereford | 37.96 | 42.00[1] | 53.03 | 49.83[1] | 90.03 | 84.34[1] |
| Solstice | 38.20 | | 48.36 | | 87.55 | |
| MDR031 (R) | 43.00 | | 45.26 | | 122.15 | |
| MDR043 (S) | 47.40 | | 45.91 | | 119.30 | |
| MDR046 (R) | 50.00 | | 45.41 | | 137.70 | |
| MDR037 (S) | 46.80 | | 47.36 | | 119.85 | |
| d.f. | 32 | 4 | 32 | 4 | 32 | 4 |
| SED | 0.749 | 0.633 | 2.191 | 1.570 | 3.294 | 2.854 |
| F statistic | | 31.50 | 7.30 | 8.05 | | 110.56 |
| F probabilty | <0.001 | <0.001 | <0.001 | 0.002 | <0.001 | <0.001 |
| Grand mean | 38.37 | 42.40 | 27.58 | 48.43 | 111.22 | 97.52 |

[1] Five varieties with *in situ* soil moisture sensors represent only four plot replicates during 2014-2015 field trial.

### 3.3.1.6 Plant root and tiller measurements of the 2013-2014 field experiment

The control variety rye indicated the highest number of seminal roots, crown roots and tillers across all varieties (Table 3.8). Within the 25 most resistant varieties, the Watkins lines and Gediflux varieties had similar ranges in the number of seminal roots and crown roots. However, the Gediflux varieties had a narrower range in the number of tillers and generally fewer tillers than the Watkins lines. The elite varieties had narrower ranges in the number of seminal and crown roots than the

Table 3.7. Correlations of crop measurements for 2013-**2014** (2014/R/WW/1419) and 2014-**2015** (2015/R/WW/1510) field trials.

| | Take-all index (0-100) | Days to anthesis from 1st May | Days to senescence from 1st June | Plant height (cm) | Number of seminal roots | Number of crown roots | Number of tillers | Total dry weights |
|---|---|---|---|---|---|---|---|---|
| Days to anthesis from 1st May | 2014: $r_s$ = 0.223, *P* = 0.059, n = 33<br>2015: $r_s$ = -0.175, *P* = 0.171, n = 5 | | | | | | | |
| Days to senescence from 1st June | 2014: $r_s$ = 0.051, *P* = 0.194, n = 33<br>2015: $r_s$ = -0.100, *P* = 0.171, n = 5 | **2014:** $r_s$ **= 0.275, *P*= 0.034, n = 33**<br>2015: $r_s$ = 0.675, *P* = 0.058, n = 5 | | | | | | |
| Plant height (cm) | 2014: $r_s$ = -0.139, *P* = 0.110, n = 33<br>2015: $r_s$ = -0.500, *P* = 0.088, n = 5 | 2014: $r_s$ = 0.141, *P* = 0.119, n = 33<br>2015: $r_s$ = -0.125, *P* = 0.196, n = 5 | **2014:** $r_s$ **= -0.428, *P* = 0.003, n = 33**<br>**2015:** $r_s$ **= -0.700, *P* = 0.033, n = 5** | | | | | |
| Number of seminal roots | **2014: $r_s$ = -0.402, *P* = 0.005, n = 33**<br>2015: $r_s$ = -0.600, *P* = 0.058, n = 5 | **2014: $r_s$ = -0.402, P = 0.005, n = 33**<br>2015: $r_s$ = -0.575, P = 0.058, n = 5 | 2014: $r_s$ = -0.108, *P* = 0.134, n = 33<br>2015: $r_s$ = -0.300, *P* = 0.129, n = 5 | **2014:** $r_s$ **= 0.396, *P* = 0.006, n = 33**<br>2015: $r_s$ = 0.300, *P* = 0.129, n = 5 | | | | |
| Number of crown roots | 2014: $r_s$ = 0.006, *P* = 0.244, n = 33<br>2015: $r_s$ = 0.100, *P* = 0.196, n = 5 | 2014: $r_s$ = 0.237, P = 0.052, n = 33<br>2015: $r_s$ = 0.675, *P* = 0.058, n = 5 | **2014: $r_s$ = 0.362, *P* = 0.010, n = 33**<br>**2015: $r_s$ = 1.000, *P* = <0.001, n = 5** | 2014: $r_s$ = -0.134, *P* = 0.114, n = 33<br>**2015: $r_s$ = -0.700, *P* = 0.033, n = 5** | **2014: $r_s$ = -0.258, *P* = 0.035, n = 33**<br>2015: $r_s$ = -0.300, *P* = 0.129, n = 5 | | | |
| Number of tillers | 2014: $r_s$ = -0.139, *P* = 0.110, n = 33<br>2015: $r_s$ = 0.300, *P* = 0.129, n = 5 | **2014: $r_s$ = 0.250, *P* = 0.045, n = 33**<br>**2015: $r_s$ = -0.675, *P* = 0.033, n = 5** | **2014: $r_s$ = -0.321, *P* = 0.017**, n = 33<br>2015: $r_s$ = 0.000, *P* = 0.237, n = 5 | **2014: $r_s$ = 0.316, *P* = 0.018**, n = 33<br>2015: $r_s$ = -0.400, *P* = 0.112, n = 5 | 2014: $r_s$ = -0.055, *P* = 0.185, n = 33<br>2015: $r_s$ = 0.400, *P* = 0.112, n = 5 | **2014: $r_s$ = 0.298, *P* = 0.023, n = 33**<br>2015: $r_s$ = 0.000, *P* = 0.237, n = 5 | | |
| Total dry weights[1] | 2014: $r_s$ = 0.100, *P* = 0.196, n = 5<br>2015: $r_s$ = -0.100, *P* = 0.196, n = 5 | 2014: $r_s$ = 0.500, *P* = 0.112, n = 5<br>2015: $r_s$ = 0.675, *P* = 0.058, n = 5 | 2014: $r_s$ = 0.300, *P* = 0.129, n = 5<br>**2015: $r_s$ = 1.000, *P* = <0.001, n = 5** | **2014: $r_s$ = -0.700, *P* = 0.033**, n = 5<br>**2015: $r_s$ = -0.700, *P* = 0.033**, n = 5 | 2014: $r_s$ = 0.600, *P* = 0.058, n = 5<br>2015: $r_s$ = -0.300, *P* = 0.129, n = 5 | 2014: $r_s$ = 0.600, *P* = 0.058, n = 5<br>**2015: $r_s$ = 1.000, *P* = <0.001, n = 5** | 2014: $r_s$ = 0.200, *P* = 0.171, n = 5<br>2015: $r_s$ = 0.000, *P* = 0.237, n = 5 | |

[1] Note: total dry weights were only carried out on the 5 varieties with inserted soil moisture sensors for both field trials.

Watkins and Gediflux varieties. The *T. monococcum* diploid accessions had similar numbers of seminal roots and crown roots to the elite varieties, however the accessions had significantly more tillers than any of the other 29 cereal varieties. There was a weakly negative correlation between the number of seminal roots and the number of crown roots ($r_s$ = -0.258, *P* = 0.035, n = 33) and a weakly positive correlation between number of crown roots and number of tillers ($r_s$ = 0.298, *P* = 0.023, n = 33) (Table 3.7).

The measurements were then correlated against the TAI and only a moderately negative correlation that was also statistically significant was found between the number of seminal roots per plant and the TAI ($r_s$ = -0.402, *P* = 0.005, n = 33) (Table 3.7). The below-ground phenotypic root measurements were then correlated against the above-ground phenotypic crop measurements (Table 3.7). A moderately positive correlation that was also statistically significant was found between the number of crown roots per day and days to senescence ($r_s$ = 0.362, *P* = 0.010, n = 33). A weakly negative correlation was found between the number of seminal roots per plant and days to anthesis ($r_s$ = -0.242, *P* = 0.034, n = 33). A moderately positive correlation was found between the number of seminal roots per plant and plant height ($r_s$ = 0.396, *P* = 0.006, n = 33). Whereas the correlation between the TAI and seminal root numbers was not observed with plant height (Table 3.7).

There was a moderately negative correlation between the number of tillers and days to leaf senescence ($r_s$ = -0.321, *P* = 0.017, n = 33) and a moderately positive correlation between the number of tillers and plant height ($r_s$ = 0.316, *P* = 0.018, n = 33) (Table 3.7). There was a weakly positive correlation between tillers and days to anthesis ($r_s$ = 0.250, *P* = 0.045, n = 33). A strong negative correlation was found between the total root dry weight of the plant sample and the plant height ($r_s$ = -0.700, *P* = 0.033, n = 5).

#### 3.3.1.7 Plant root and tiller measurements of the 2014-2015 field experiment

The below-ground phenotypic root measurements and plant tiller counts were analysed for the five varieties with *in situ* soil moisture sensors in the four field plots (Table 3.8). There were significant effects of cereal varieties on the number of

seminal roots ($P$ = <0.001) and the total root dry weights of the plant samples ($P$ = 0.036) (Table 3.8). There were no significant effects of cereal varieties on the number of crown roots ($P$ = 0.647) or number of tillers ($P$ = 0.296) (Table 3.8). There was a trend for varieties to have more seminal roots in the 2015 field trial when compared to the 2014 field trial data. There was also a significant correlation in the ranking order of the number of seminal roots across the two years amongst the five varieties ($r_s$ = 0.700, $P$ = 0.033, n = 5). There was a trend for varieties to have more crown roots and more tillers in the 2014 field trial than in the trial conducted in 2015. There was no significant correlation in the ranking order of the number of crown roots across the two years amongst the five varieties ($r_s$ = 0.200, $P$ = 0.171, n = 5), although there was a significant positive correlation in the ranking order of the number of tillers across the two years amongst the varieties ($r_s$ = 0.700, $P$ = 0.033, n = 5). The varieties had a heavier root dry weight in 2014 than in 2015. The ranking order of root dry weights was however consistent across the two years amongst the five varieties ($r_s$ = 0.900, $P$ = 0.004, n = 5). There was a strong positive correlation between the total dry weight and number of crown roots ($r_s$ = 1.000, $P$ = <0.001, n = 5) (Table 3.7).

The measurements were then correlated against the TAI but no significant correlations were found (Table 3.7). The below-ground phenotypic root measurements were then correlated against the above-ground phenotypic crop measurements (Table 3.7). A strong positive correlation was found between the number of crown roots and days to senescence ($r_s$ = 1.000, $P$ = <0.001, n = 5) and a strong negative correlation between the number of crown roots and plant height ($r_s$ = -0.700, $P$ = 0.033, n = 5). There was a strong negative correlation between tillers and days to anthesis ($r_s$ = -0.675, $P$ = 0.033, n = 5). There was a strong positive correlation between the total root dry weight and days to senescence ($r_s$ = 1.000, $P$ = <0.001, n = 5). A strong negative correlation was found between the total root dry weight and plant height ($r_s$ = -0.700, $P$ = 0.033, n = 5).

Table 3.8. Root counts, tiller counts and total root dry weights per plant of cereal varieties in the 2013-2014 (2014/R/WW/1419) and 2014-2015 (2015/R/WW/1510) field trials.

| Treatment | Number of seminal roots per plant | | Number of crown roots per plant | | Number of tillers per plant | | Total root dry weight | |
|---|---|---|---|---|---|---|---|---|
| | Square root transformation (BT[1] means) | | Square root transformation (BT[1] means) | | Square root transformation (BT[1] means) | | | |
| | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** |
| **Controls** | | | | | | | | |
| Rye | 2.39 (5.71) | | 6.54 (42.77) | | 2.27 (5.15) | | | |
| Triticale | 2.34 (5.48) | | 5.62 (31.58) | | 1.84 (3.39) | | | |
| Hereward | 2.12 (4.49) | 4.90[2] | 5.46 (29.81) | 22.77[2] | 1.93 (3.72) | 2.52 | 0.388[3] | 0.159[2] |
| Paragon | 2.02 (4.08) | 4.72[2] | 5.82 (33.87) | 21.41[2] | 1.71 (2.92) | 1.66 | 0.304[3] | 0.141[2] |
| **Varieties/lines** | | | | | | | | |
| G16 M. Tempo | 2.34 (5.48) | | 5.75 (33.06) | | 1.71 (2.92) | | | |
| G40 Marco | 2.24 (5.02) | | 5.28 (27.88) | | 1.78 (3.17) | | | |
| G51 Kontrast | 2.18 (4.75) | | 5.14 (26.42) | | 1.72 (2.96) | | | |
| 20 | 2.22 (4.93) | | 4.77 (22.75) | | 1.85 (3.42) | | | |
| 114 | 2.23 (4.97) | | 5.46 (29.81) | | 2.03 (4.12) | | | |
| 151 | 2.17 (4.71) | | 5.63 (31.70) | | 2.32 (5.38) | | | |
| 156 | 2.12 (4.49) | | 5.04 (25.40) | | 1.92 (3.69) | | | |
| 168 | 2.05 (4.20) | | 5.00 (25.00) | | 1.70 (2.89) | | | |
| 197 | 2.23 (4.97) | | 5.67 (32.15) | | 1.89 (3.57) | | | |
| 202 | 2.20 (4.84) | | 5.60 (31.36) | | 1.74 (3.03) | | | |

Table 3.8. Continued.

| | **Number of seminal roots per plant** Square root transformation (BT[1] means) | | **Number of crown roots per plant** Square root transformation (BT[1] means) | | **Number of tillers per plant** Square root transformation (BT[1] means) | | **Total root dry weight** | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** |
| **Varieties/lines** | | | | | | | | |
| 204 | 2.17 (4.71) | | 5.40 (29.16) | | 2.19 (4.80) | | | |
| 487 | 2.28 (5.20) | | 5.15 (26.52) | | 2.08 (4.33) | | | |
| 488 | 2.23 (4.97) | | 5.45 (29.70) | | 2.06 (4.24) | | | |
| 489 | 2.14 (4.58) | | 5.63 (31.70) | | 2.25 (5.06) | | | |
| 492 | 2.24 (5.02) | | 5.66 (32.04) | | 2.03 (4.12) | | | |
| 500 | 2.23 (4.97) | | 5.34 (28.52) | | 1.83 (3.35) | | | |
| 739 | 2.26 (5.11) | | 4.98 (24.80) | | 1.83 (3.35) | | | |
| 776 | 2.25 (5.06) | | 5.27 (27.77) | | 1.99 (3.96) | | | |
| 777 | 2.14 (4.58) | 6.43[2] | 5.77 (33.29) | 19.36[2] | 1.91 (3.65) | 2.37[2] | 0.225[3] | 0.078[2] |
| 794 | 2.23 (4.97) | | 5.31 (28.20) | | 2.25 (5.06) | | | |
| 821 | 2.14 (4.58) | | 5.85 (34.22) | | 2.14 (4.58) | | | |
| Cassius | 2.04 (4.16) | | 5.94 (35.28) | | 1.87 (3.50) | | | |
| Duxford | 2.05 (4.20) | 5.36[2] | 5.99 (35.88) | 22.95[2] | 1.72 (2.96) | 1.89[2] | 0.522[3] | 0.169[2] |
| Hereford | 2.13 (4.54) | 5.56[2] | 6.04 (36.48) | 22.13[2] | 2.03 (4.12) | 2.04[2] | 0.438[3] | 0.147[2] |
| Solstice | 2.18 (4.75) | | 5.40 (29.16) | | 1.69 (2.86) | | | |

Table 3.8. Continued.

| | Number of seminal roots per plant Square root transformation (BT[1] means) | | Number of crown roots per plant Square root transformation (BT[1] means) | | Number of tillers per plant Square root transformation (BT[1] means) | | Total root dry weight | |
|---|---|---|---|---|---|---|---|---|
| **Treatment** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** | **2014** | **2015** |
| **Varieties/lines** | | | | | | | | |
| MDR031 (R) | 2.12 (4.49) | | 5.76 (33.18) | | 3.59 (12.89) | | | |
| MDR043 (S) | 1.96 (3.84) | | 5.76 (33.18) | | 3.43 (11.76) | | | |
| MDR046 (R) | 2.12 (4.49) | | 5.52 (30.47) | | 3.27 (10.69) | | | |
| MDR037 (S) | 2.20 (4.84) | | 6.17 (38.07) | | 3.29 (10.82) | | | |
| d.f. | 32 | 4 | 32 | 4 | 32 | 4 | 4 | 4 |
| SED | 0.067 | 0.254 | 0.27 | 2.575 | 0.168 | 0.421 | 0.082 | 0.027 |
| F statistic | | 14.12 | | 0.64 | | 1.38 | 4.15 | 3.56 |
| F probabilty | <0.001 | <0.001 | <0.001 | 0.647 | <0.001 | 0.296 | 0.02 | 0.036 |
| Grand mean | 2.17 (4.71) | 5.39 | 5.53 (30.58) | 21.72 | 2.08 (4.33) | 2.10 | 0.375 | 0.139 |

[1] BT = back transformed means.

[2] Five varieties with *in situ* soil moisture sensors represent only four plot replicates during 2014-2015 field trial.

[3] Total root dry weight data for only the four plot replicates with *in situ soil* moisture sensors during 2013-2014 field trial.

### 3.3.2 Soil moisture performance under take-all susceptible and moderately resistant hexaploid wheat varieties in the field

A number of problems arose with the soil moisture sensors throughout the two field seasons, leading to a number of gaps in the two datasets and the need for a number of replacement cables and sensors.

#### 3.3.2.1 Soil moisture measurements of the 2013-2014 field experiment

During the 2013-2014 field season, *in situ* soil moisture sensors recorded the soil water content from 22nd November 2013 to 11th July in four plots for each of the varieties Hereward, Paragon, Duxford and Watkins line 777, three plots for the variety Hereford and one fallow plot as well as a soil temperature sensor inserted into a separate Hereward plot (Figure 3.5) (see Appendix 3.1 for field plan). The average soil temperature ranged from 2.7°C - 20.4°C throughout the 2014 field trial season (Figure 3.5). The daily soil water content was plotted against the daily rainfall data, downloaded from the e-RA, to indicate rainfall events and to compare the level of soil drying after and between such events (Figure 3.5). High and extremely variable soil water content was recorded throughout the winter months and at the beginning of spring for all varieties. The soil water content could then be seen to become more stable and rapidly declined from 3rd March 2014 and throughout the remainder of the growing season, with the exceptions of major rainfall events. Peaks in soil water content could be seen directly after each of the major rainfall events during the field season: 23rd December, 1st January, 31st January, 6th February, and 27th May. Interestingly, you could also see a dip in the average soil temperature after each of the major rainfall events.

The daily soil water content was then plotted against the potential soil water deficit (PSMD) to compare the level of soil moisture with the water availability in the field trial site (Figure 3.6). The PSMD can be seen to mirror the rainfall and is at field capacity (PSMD = 0) from 5th January, after two signficant rainfall events on the 23rd December and 1st January, until 5th March (Figure 3.6). The PSMD then began to steadily increase whilst

being responsive to rainfall. The soil water content of the individual sensors illustrated more of a stable drying effect, indicating that the soil water content was not as sensitive to the rainfall events as the PSMD calculation. The PSMD can then be seen to rapidly increase from 20.1 on 28th May, after the last major rainfall event on 27th May, up to 109.2 by the end of measurement on 11th July.

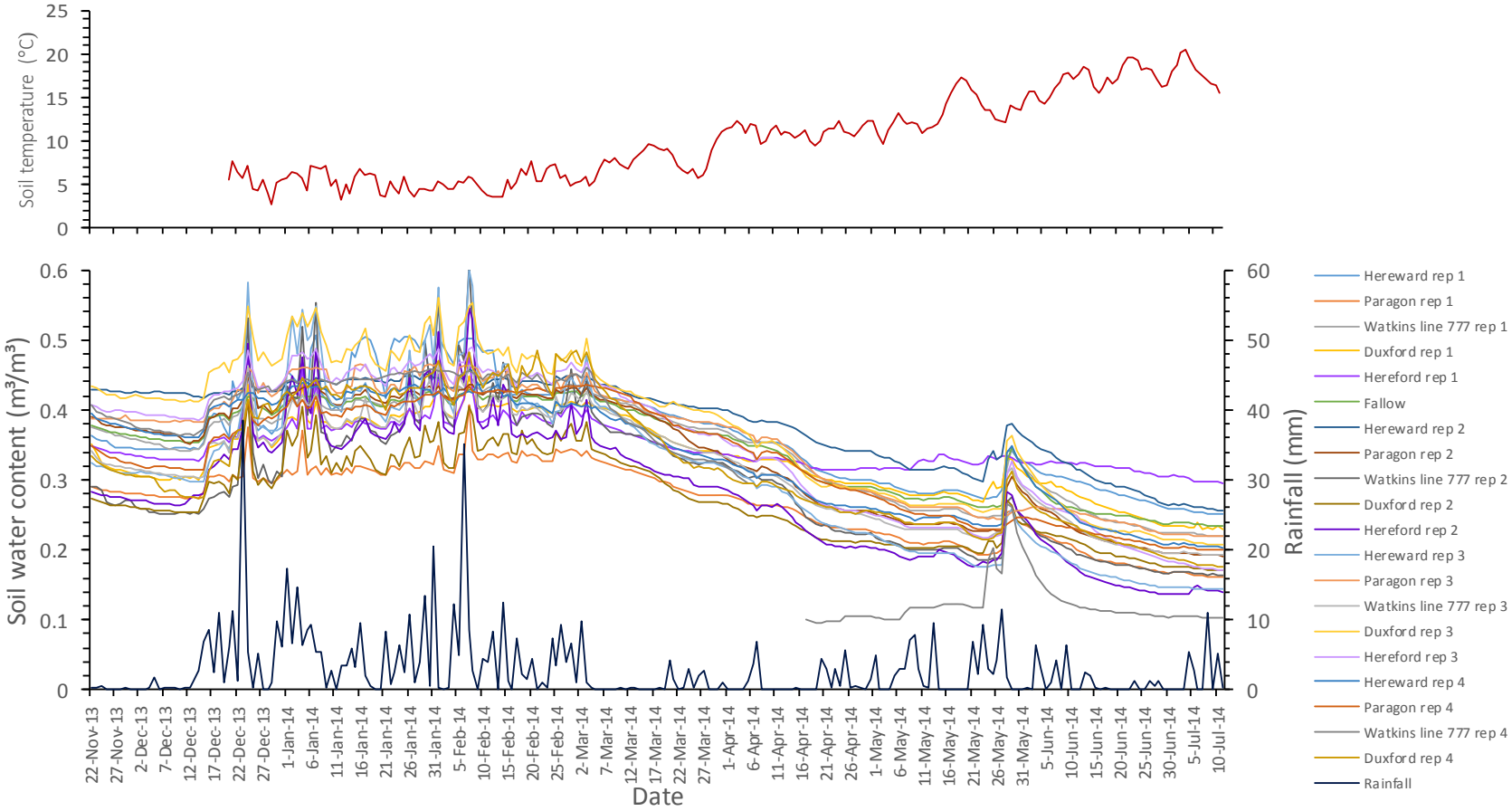



**Figure 3.5.** Daily soil temperature (°C) (top) and daily soil water content (m³ water/m³ soil), both recorded at a depth of 15 cm, for five varieties and fallow plot of the 2013-2014 (2014/R/WW/1419) field trial plotted against daily rainfall (mm) (bottom) from 22/11/13 - 11/07/14.

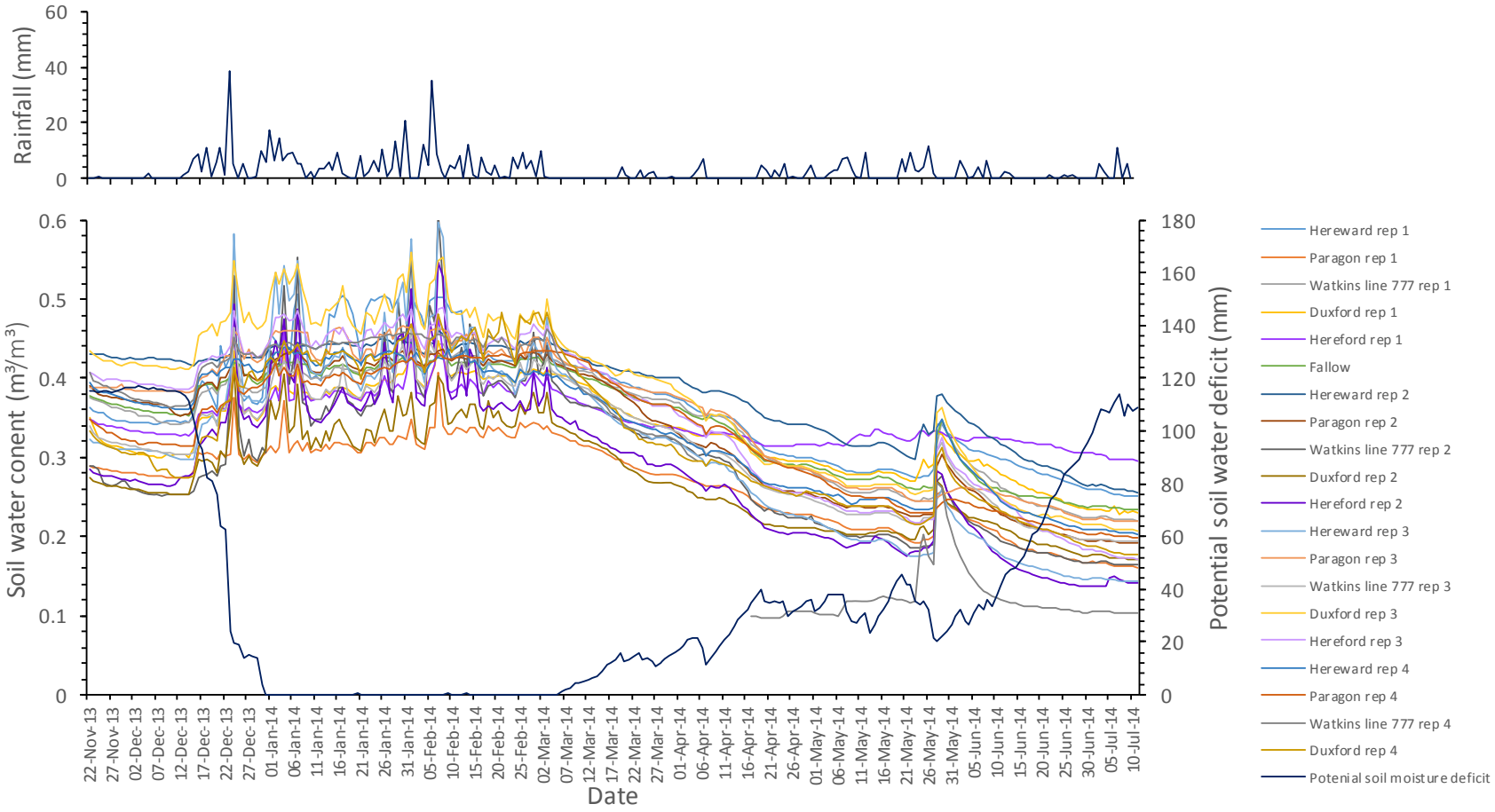



**Figure 3.6.** Daily rainfall (mm) (top) and daily soil moisture ($m^3$ water/$m^3$ soil) recorded at a depth of 15 cm for five varieties and fallow plot of the 2013-2014 (2014/R/WW/1419) field trial plotted against the potential soil water deficit (mm) (bottom) from 22/11/13 - 11/07/14.

There was a large amount of variability between individual soil moisture sensors over the timecourse of the 2014 field trial (Figure 3.5). The degree of saturation for the soil water content data was therefore calculated to help normalise the data, yet variability in the saturation measurements were still seen between replicates for each variety (Figure 3.7 & Figure 3.8). The majority of varieties were virtually at complete saturation until the beginning of March, when the soil began to dry. The single fallow plot indicated the highest degree of saturation compared to all of the varieties except the take-all susceptible control variety Hereward, where replicate 2 was found to have the highest degree of saturation as well as the highest TAI from the four replicates (Figure 3.7 & Figure 3.8). Out of the four Hereward replicates, replicate 3 indicated a lower degree of saturation throughout the spring and summer months (Figure 3.7). The second take-all susceptible control variety Paragon, also indicated that one of the replicates had a much lower degree of saturation throughout March-July (Figure 3.7). The Paragon replicates 3 and 4 did not appear to respond in their level of saturation as much as replicate 1, replicate 2 or the fallow plot to the rainfall events in late May. The degree of saturation of three of the replicates of the moderately take-all resistant variety Duxford were comparable, whereas the degree of saturation of replicate 1 was closer to that of the fallow plot (Figure 3.8). However, a large amount of variation in the degree of saturation was found between the four replicates of the take-all resistant variety Hereford (Figure 3.8). Hereford replicate 1 revealed a consistent level of saturation throughout March-July with no indication of soil drying. The take-all resistant Watkins line 777 also indicated a large amount of variation in the degree of saturation amongst the four replicates (Figure 3.8). The degree of saturation for Watkins line 777 replicate 3 and replicate 1 were more similar to the fallow plot, whereas replicate 2 and replicate 4 had a much lower level of saturation, the degree of saturation appeared to be independent of TAI score.

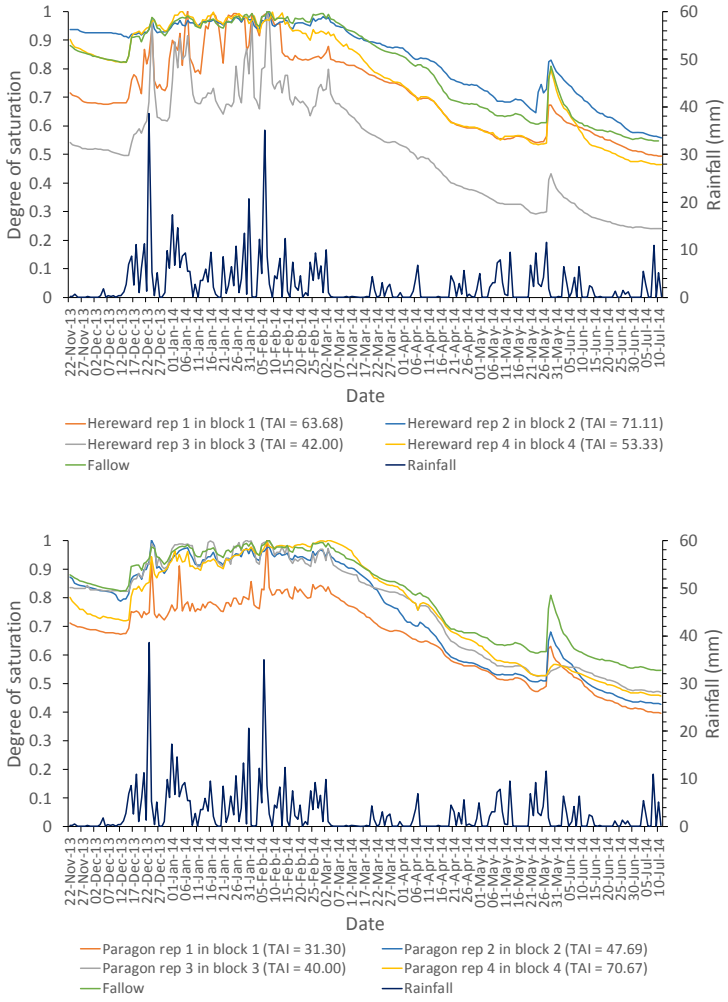



**Figure 3.7.** Degree of saturation from the four plot replicates of the susceptible control varieties and one fallow plot of variety Hereward (top) and variety Paragon (bottom) for the 2013-2014 (2014/R/WW/1419) field trial plotted against daily rainfall (mm) from 22/11/13 - 11/07/14. Field trial experimental block and take-all index (TAI) indicated next to each plot replicate.

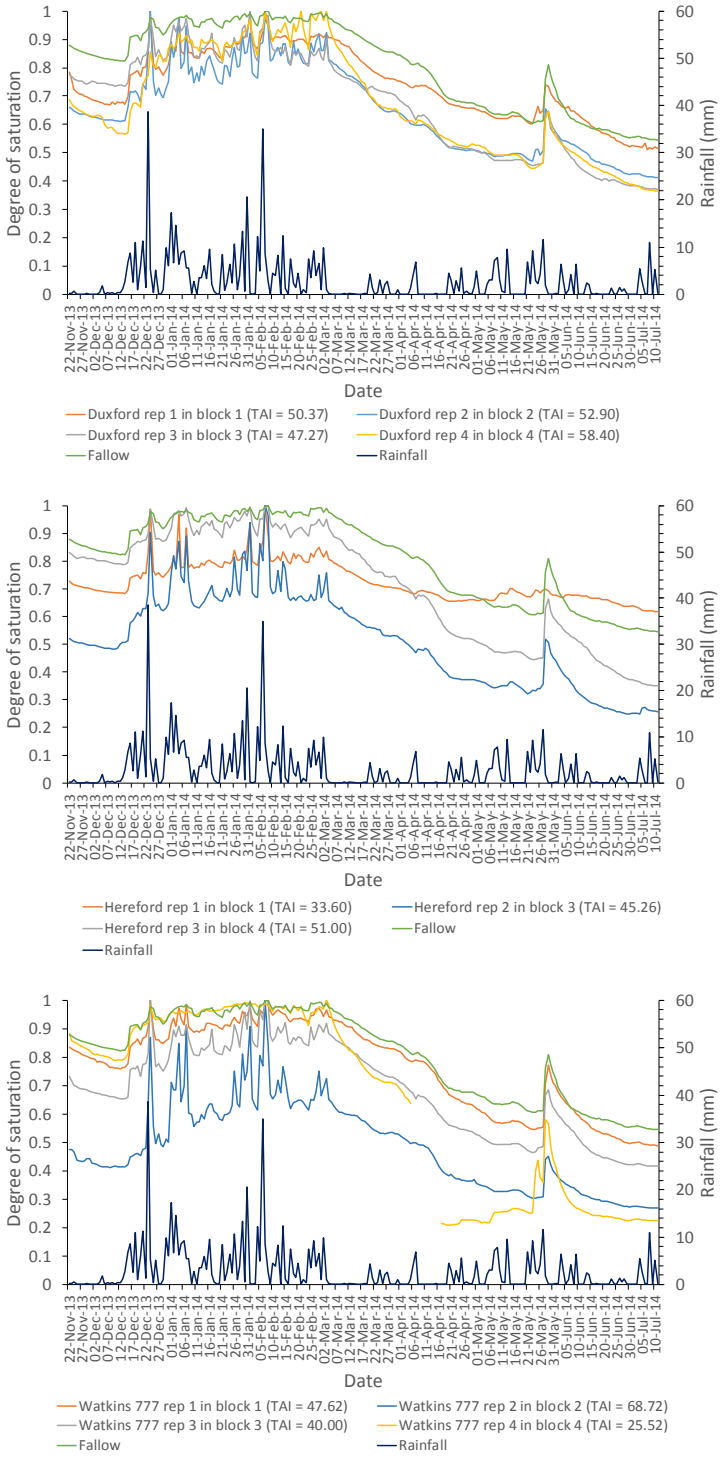



**Figure 3.8.** Degree of saturation from the four plot replicates of the moderately resistant varieties and fallow plot of variety Duxford (top), variety Hereford (middle) and Watkins line 777 (bottom) for the 2013-2014 (2014/R/WW/1419) field trial plotted against daily rainfall (mm) from 22/11/13 - 11/07/14. Field trial experimental block and take-all index (TAI) indicated next to each plot replicate.

The degree of saturation of each plot replicate was then plotted against the TAI for the individual plot replicates for each variety. There was a large amount of variation in the TAI scores across the experimental blocks, especially for the two susceptible varieties Hereward and Paragon as well as the moderately resistant Watkins line 777 (Figure 3.9 & Figure 3.10). As shown previously in Table 3.5, the three moderately resistant varieties had no less disease than the susceptible varieties.

The ranking of the TAI score across the blocks for each variety indicated that the TAI was highest for the plot replicate in block 4 for the varieties of Paragon, Duxford and Hereford and highest for the plot replicate in block 2 for the varieties Hereward and Watkins line 777 (Figure 3.9 & Figure 3.10). A linear relationship was found between the degree of saturation and the TAI for three of the four plot replicates of the take-all susceptible variety Hereward, the higher the degree of saturation the higher the TAI (Figure 3.9). The lowest degree of saturation recorded across the four dates correlated with the lowest TAI for the Hereward plot replicate in block 3 of the experimental field trial design and the highest degree of saturation across the four dates correlated with the highest TAI for the Hereward plot replicate in block 2 (Figure 3.9). Less of a relationship was found between the degree of saturation and the TAI for the take-all susceptible variety Paragon (Figure 3.9). The plot replicate in block 3 had the highest degree of saturation across two of the four dates, yet had the second lowest TAI of the four plot replicates. There appeared to be no relationship between the degree of saturation and the TAI for the take-all moderately resistant varieties of Duxford and Hereford (Figure 3.10). The TAI was fairly consistent across the four Duxford plot replicates (Figure 3.10). There were only three plot replicates for the Hereford variety and the replicate in block 1 had a consistent level in the degree of saturation across the entire season, as seen in Figure 3.8 making it difficult to establish the plots relationship with the level of take-all disease presence (Figure 3.10). There appeared to be a linear relationship between the degree of saturation and

the TAI for three of the four plot replicates of the take-all resistant Watkins line 777 (Figure 3.10). The plot replicate in block 2 of the experimental field trial design, had a low degree of saturation from the initial recording at the start of the field season and a low level of saturation throughout the rest of the year (Figure 3.8). Ideally the soil moisture sensor would have been removed and re-inserted to enable the soil moisture recordings to be more consistent with the other three replicates.

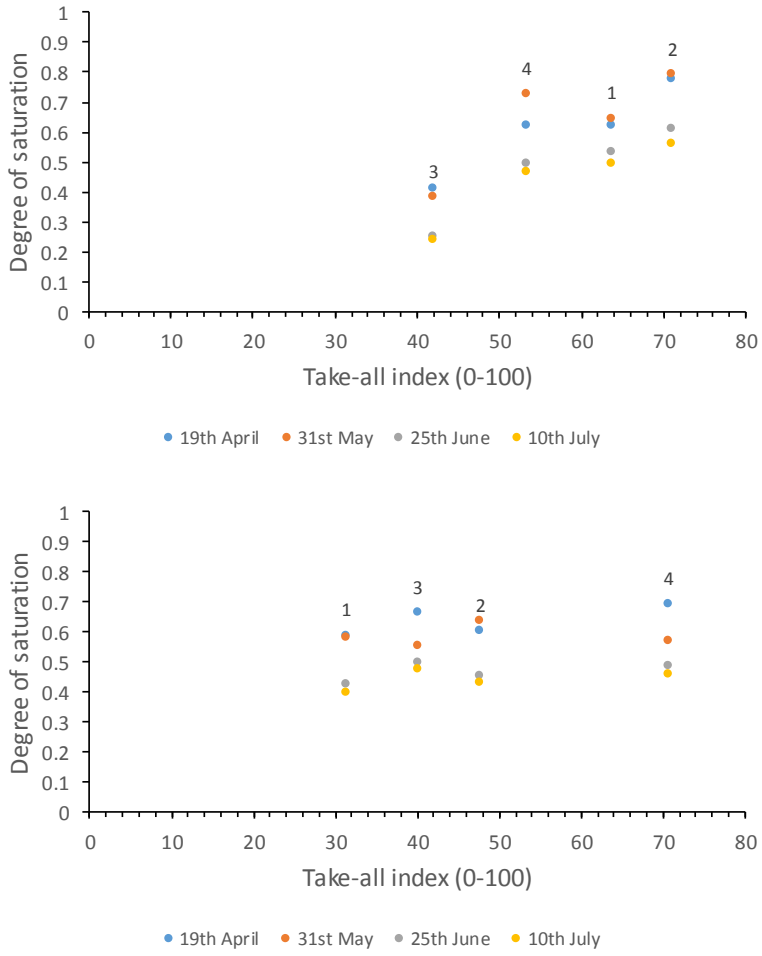



**Figure 3.9**. Degree of saturation for four plot replicates (experimental blocks numbered above) of the take-all susceptible varieties Hereward (top) and Paragon (bottom) plotted against the take-all index (TAI) for 19th April, 31st May, 25th June and 10th July of the 2013-2014 (2014/R/WW/1419) field trial.

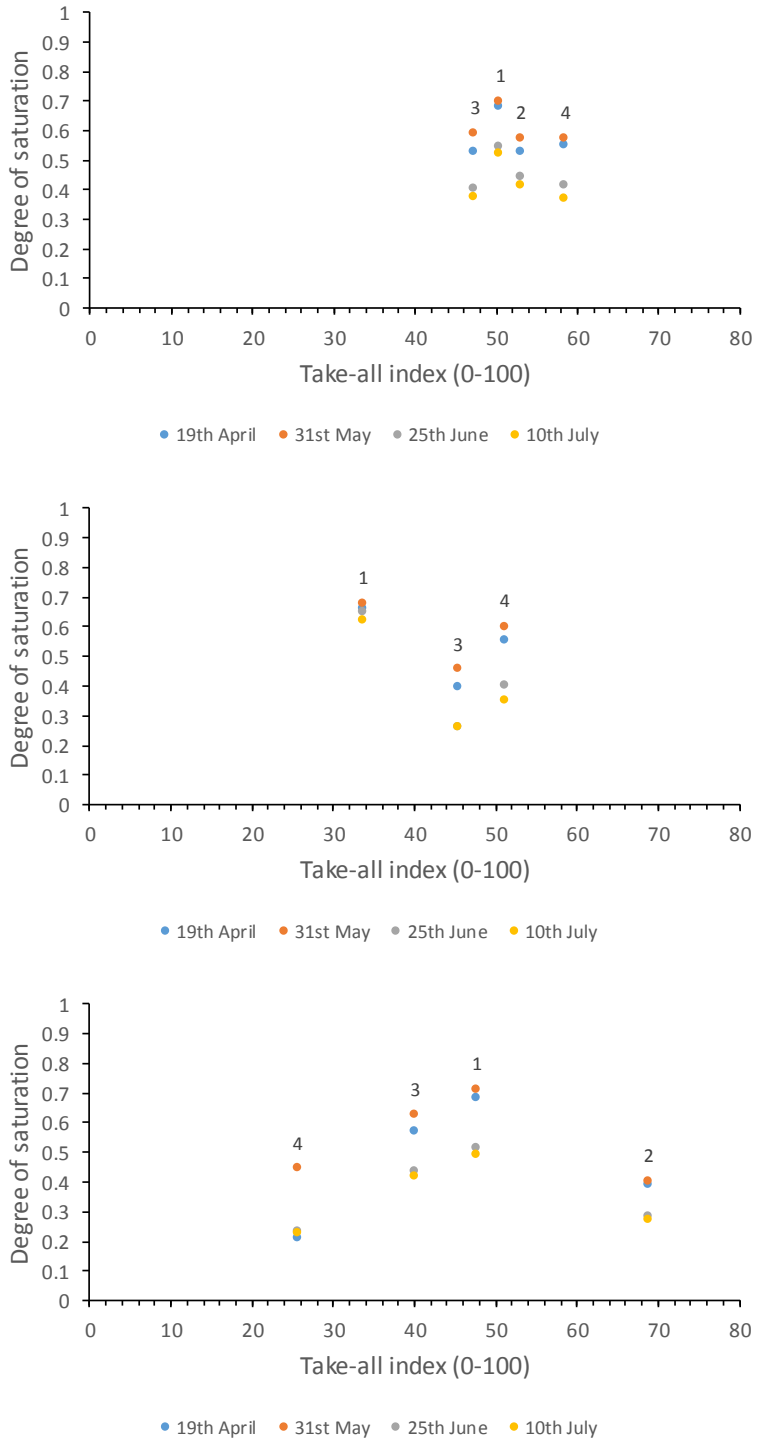



**Figure 3.10.** Degree of saturation for three or four plot replicates (experimental blocks numbered above) of the take-all moderately resistant varieties Duxford (top), Hereford (middle) and Watkins line 777 (bottom) plotted against the take-all index (TAI) for 19th April, 31st May, 25th June and 10th July of the 2013-2014 (2014/R/WW/1419) field trial.

The relationship between the TAI and the degree of saturation was further explored by plotting the degree of saturation after a major rainfall event to look at the difference in the level of soil drying across the varieties. There was a major rainfall event on 27th May 2014, a total daily rainfall of 11.5 mm (see Figure 3.5), and the next six consecutive days were plotted against the TAI for each individual plot (Figure 3.11 & Figure 3.12). There appeared to be a slight trend for the two Hereward plot replicates with the highest level of take-all infection to have less of a spread in the degree of saturation measurements across the six dates and therefore less soil drying from the initial date of 28th May to the final date of 2nd June than the two plot replicates with a lower TAI (Figure 3.11). No trend in the level of soil drying was found across the four Paragon plot replicates (Figure 3.11). A similar level of soil drying was found across all four replicates of the moderately resistant variety Duxford; this was expected due to the four replicates illustrating similar TAI scores (Figure 3.12). The replicate of the moderately resistant variety Hereford in experimental block one, demonstrated very little soil drying post the major rainfall event on the 27th May even though this replicate had the lowest TAI score (Figure 3.12). The opposite trend was found for the replicate with the lowest TAI score of the moderately resistant Watkins line 777, with the replicate illustrating a large amount of soil drying from the initial date of 28th May to the final date of 2nd June (Figure 3.12). Due to the similar TAI scores of the five varieties, it is difficult to compare the levels of soil drying between the moderately resistant take-all varieties and the take-all susceptible varieties.

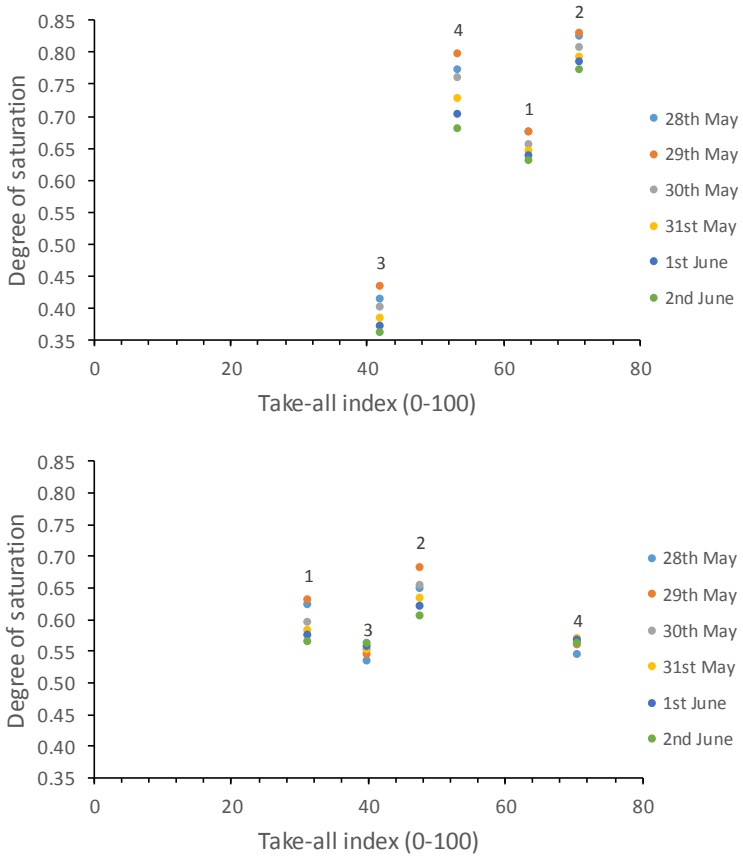



**Figure 3.11.** The level of soil drying after a major rainfall event on the 27th May 2014. The degree of saturation for the four plot replicates (experimental blocks numbered above) of the take-all susceptible varieties Hereward (top) and Paragon (bottom) plotted against the take-all index (TAI) for six consecutive days after the 27th May of the 2013-2014 (2014/R/WW/1419) field trial.

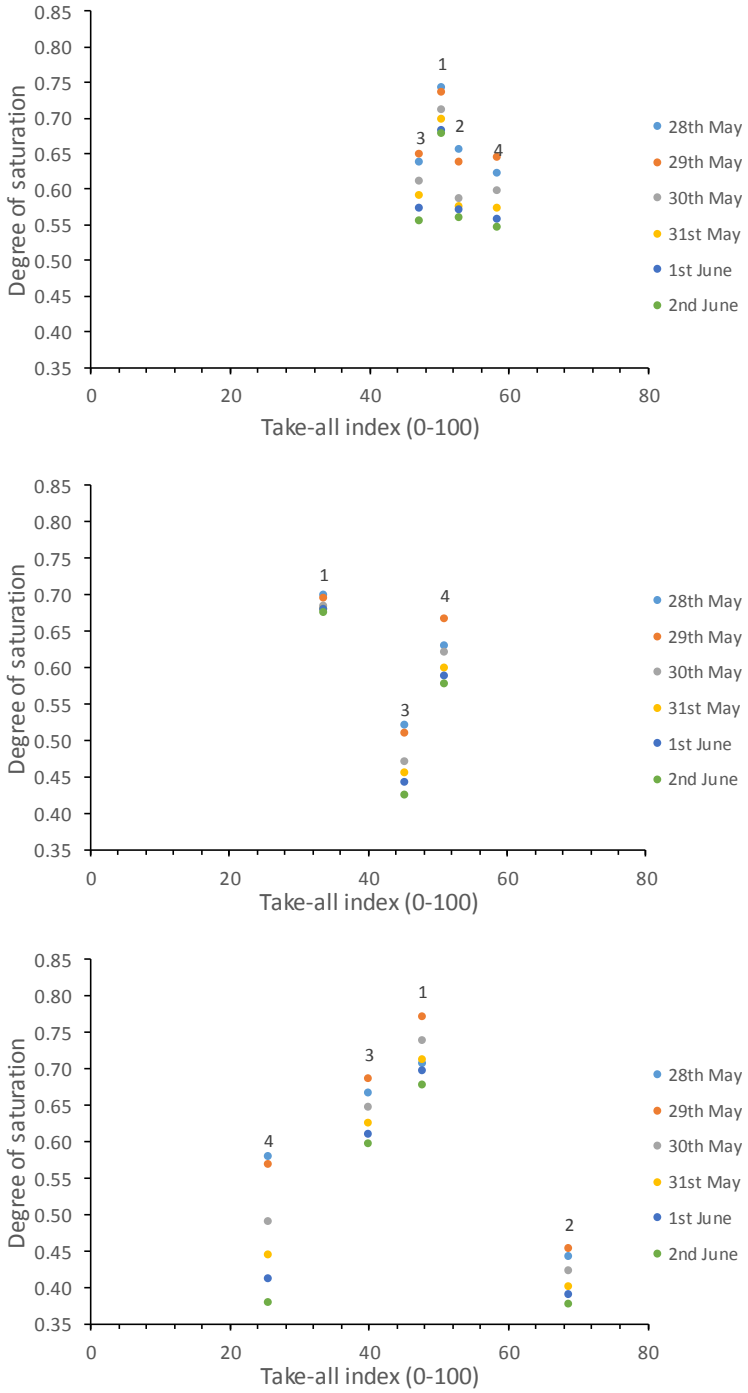



**Figure 3.12.** The level of soil drying after a major rainfall event on the 27th May 2014. The degree of saturation for the three or four plot replicates (experimental blocks numbered above) of the take-all moderately resistant varieties Duxford (top), Hereford (middle) and Watkins line 777 (bottom) plotted against the take-all index (TAI) for six consecutive days after the 27th May of the 2013-2014 (2014/R/WW/1419) field trial.

#### 3.3.2.2 Soil moisture measurements of the 2014-2015 field experiment

Within the 2014-2015 field season, *in situ* soil moisture sensors recorded the soil water content from 3rd December 2013 to 10th August 2015 in four plots for each of the varieties Hereward, Paragon, Duxford, Hereford and Watkins line 777 and two fallow areas in the discard area north and south of the field trial as well as a soil temperature sensor inserted into a separate Hereward plot (Figure 3.13) (see Appendix 3.1 for field plan). Throughout the 2015 field trial, the average daily soil temperature ranged from 1.41°C - 21.39°C (Figure 3.13).

As seen with the 2013-2014 field trial, the daily soil water content was high and variable for all varieties throughout the winter months of the 2015 field trial (Figure 3.5 & Figure 3.13). Peaks in the soil water content were found for each sensor after each of the major rainfall events during the winter months. Unfortunately, there was a failure of both leisure batteries for each of the two data loggers from 11th January - 20th February (Figure 3.13). The battery in the south box also failed from 11th March - 1st April (Figure 3.13). The soil water content became less variable and began declining from around 1st March 2015 although an increase in the soil water content was found for the majority of individual sensors after the major rainfall events: 11th December, 16th December, 26th December, 22nd February, 26th February, 4th May, 14th May, 3rd July, 16th July, 24th July and 26th July (Figure 3.13).

The daily soil water content was then plotted against the PSMD (Figure 3.14). The PSMD could be seen to mirror the rainfall and was at field capacity (PSMD = 0) from 3rd December, with the exception of reaching between 0.3 - 0.7 on five dates, until 2nd March. The trial reached field capacity a month earlier than found during the 2013-2014 field trial season (Figure 3.6 & Figure 3.14); almost double the amount of rain fell in November 2014 than November 2015 (Table 3.4). The soil moisture deficit then began to increase from 2nd March onwards, with the exception of temporarily decreasing after rainfall events (Figure 3.14). A higher number

of major rainfall events occurred during the 2014-2015 field trial seaon from March onwards. The soil water content recorded for the majority of the individual sensors were found to be more sensitive to the major rainfall events, from the 2nd March onwards, than found in the same time period during the 2014 field trial (Figure 3.6 & Figure 3.14).

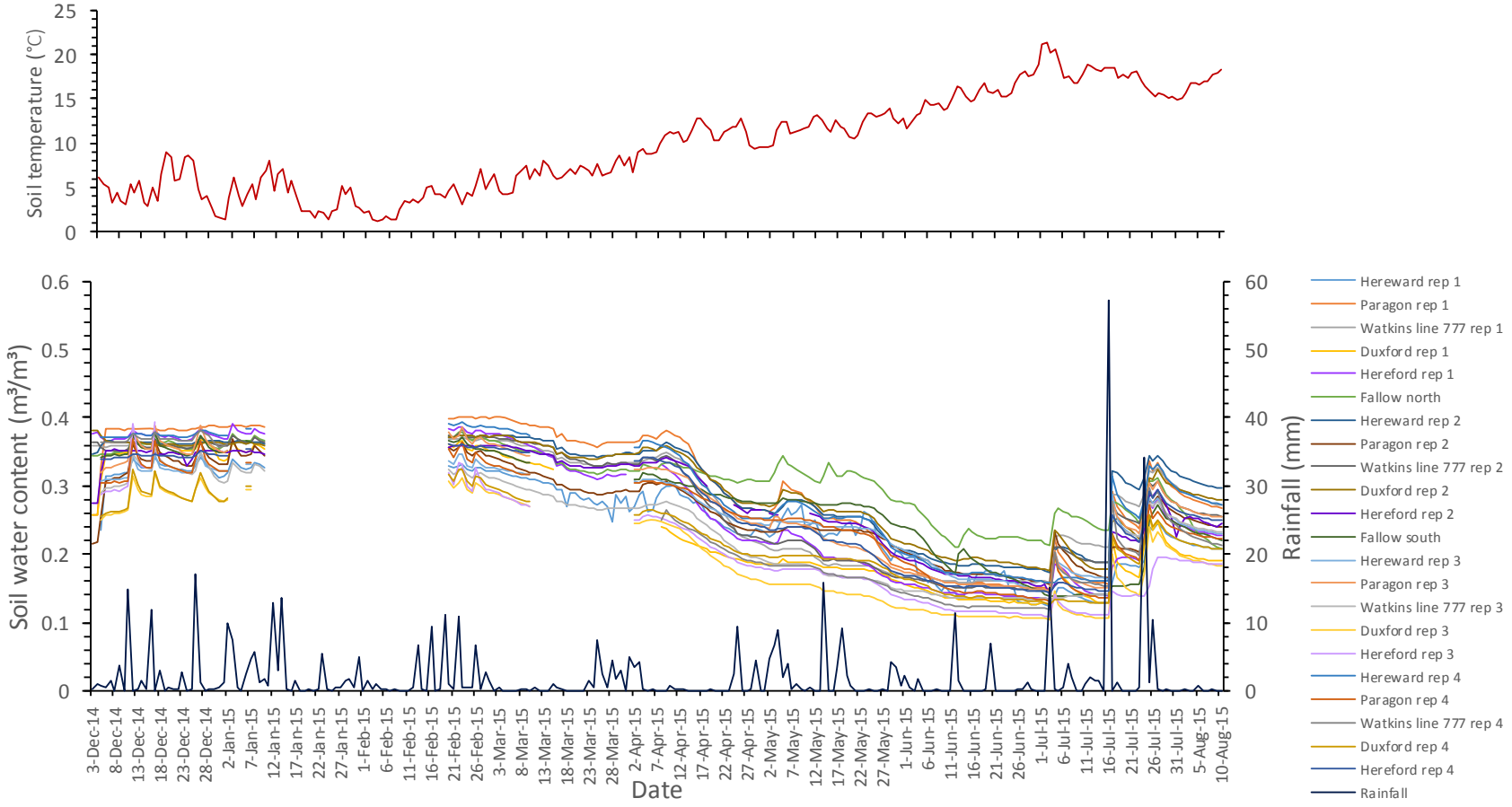



**Figure 3.13.** Daily soil temperature (°C) (top) and daily soil water content (m³ water/m³ soil, both recorded at a depth of 15 cm, for five varieties and fallow plot of the 2014-2015 (2015/R/WW/1510) field trial plotted against daily rainfall (mm) (bottom) from 03/12/14 - 10/08/15.

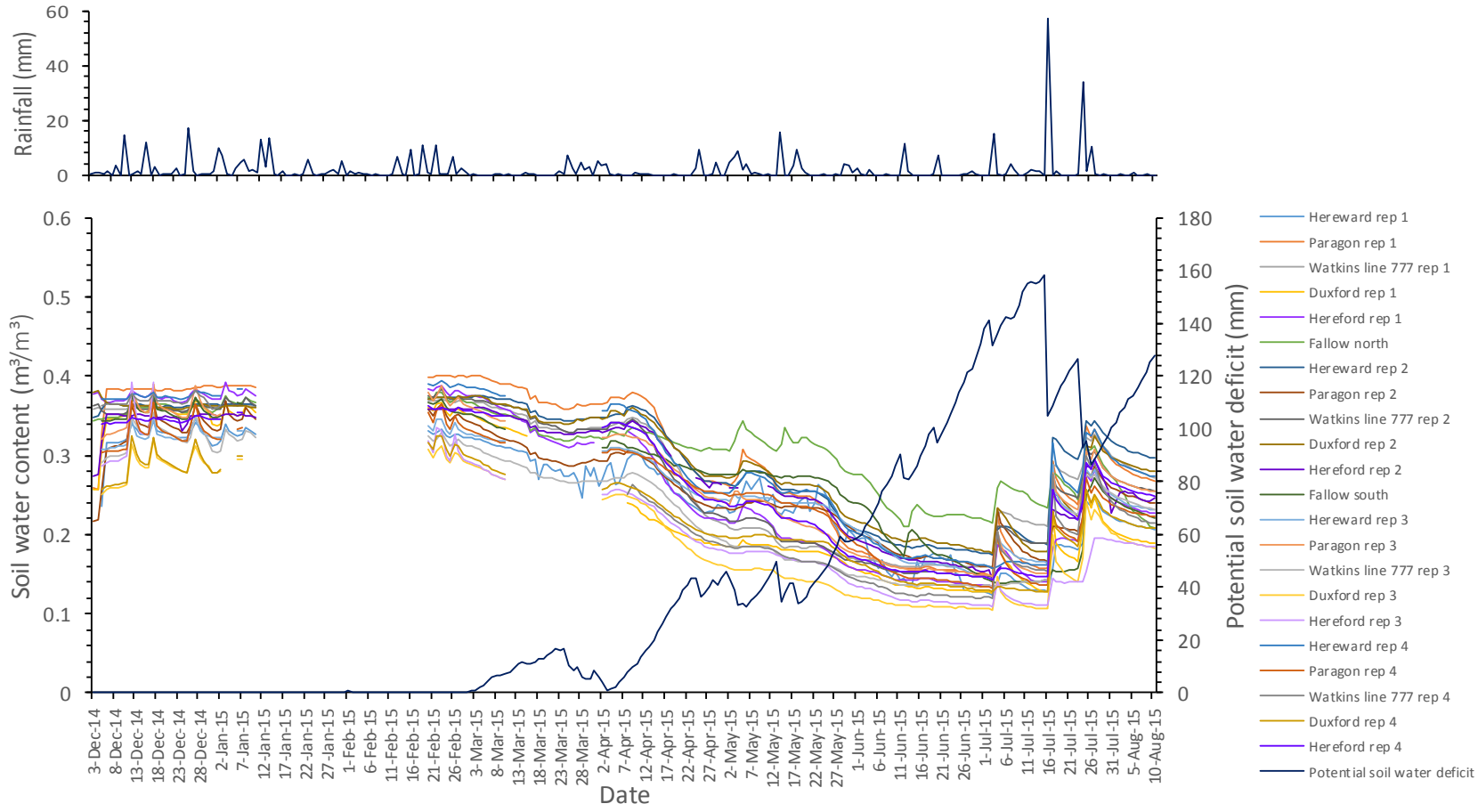



**Figure 3.14.** Daily rainfall (mm) (top) and daily soil moisture (m³ water/m³ soil) recorded at a depth of 15 cm for five varieties and two fallow plots of the 2014-2015 (2015/R/WW/1510) field trial plotted against potential soil water deficit (mm) (bottom) from 03/12/14 - 10/08/15.

The degree of saturation for the soil water content was calculated to normalise the data as seen for the 2013-2014 field trial. Variability was still found between the plot replicates in the saturation measurements for each variety. Complete saturation was found for all varieties during the winter months. The varieties began to decrease the amount of soil moisture from early/mid-March. The two fallow plots indicated the highest degree of saturation compared to the majority of the plot replicates of each variety. There was slight variation in the level of saturation between the two fallow plots, although the two plots indicated the same trend with the exception of the north fallow plot not responding to the rain event on the 3rd July. The plot replicates of the take-all susceptible control variety Hereward were fairly similar in their level of saturation from April onwards, however the saturation measurements of replicate 1 appeared to be more noisy in response to rainfall events (Figure 3.15). The four replicates of the take-all susceptible variety Paragon were comparable in their level of saturation throughout the season, with the exception of replicate 1 starting at a higher degree of stauration (Figure 3.15). There was a large amount of variation in the degree of saturation across the four replicates of the take-all moderately resistant variety Duxford (Figure 3.16). Duxford replicate 2 had a similar level of saturation as the two fallow plots during the spring to early summer months. A large amount of variation in the level of saturation was also found with the take-all resistant variety Hereford, as seen with the 2014 field trial measurements (Figure 3.8 & Figure 3.16). Hereford replicate 3 had a lower soil water content from intial recordings during the winter months (Figure 3.13) and therefore a lower level of saturation (Figure 3.16). The degree of saturation of the four replicates of the take-all resistant Watkins line 777 were fairly comparable (Figure 3.16). The degree of saturation was a lot lower than the two fallow plots for all of the Watkins line 777 replicates from late April to early June.

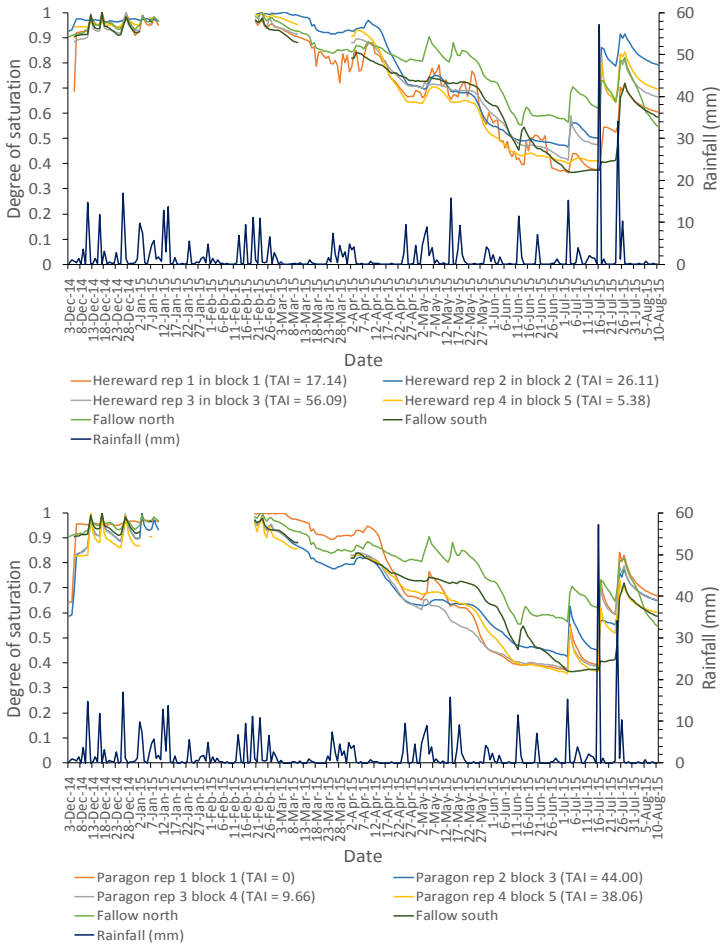



**Figure 3.15.** Degree of saturation from the four plot replicates of the susceptible control varieies and two fallow plot replicates for variety Hereward (top) and variety Paragon (bottom) for the 2013-2014 field trial plotted against daily rainfall (mm) from 03/12/14 - 10/08/15. Field trial experimental block number and take-all index (TAI) indicated next to each plot replicate.

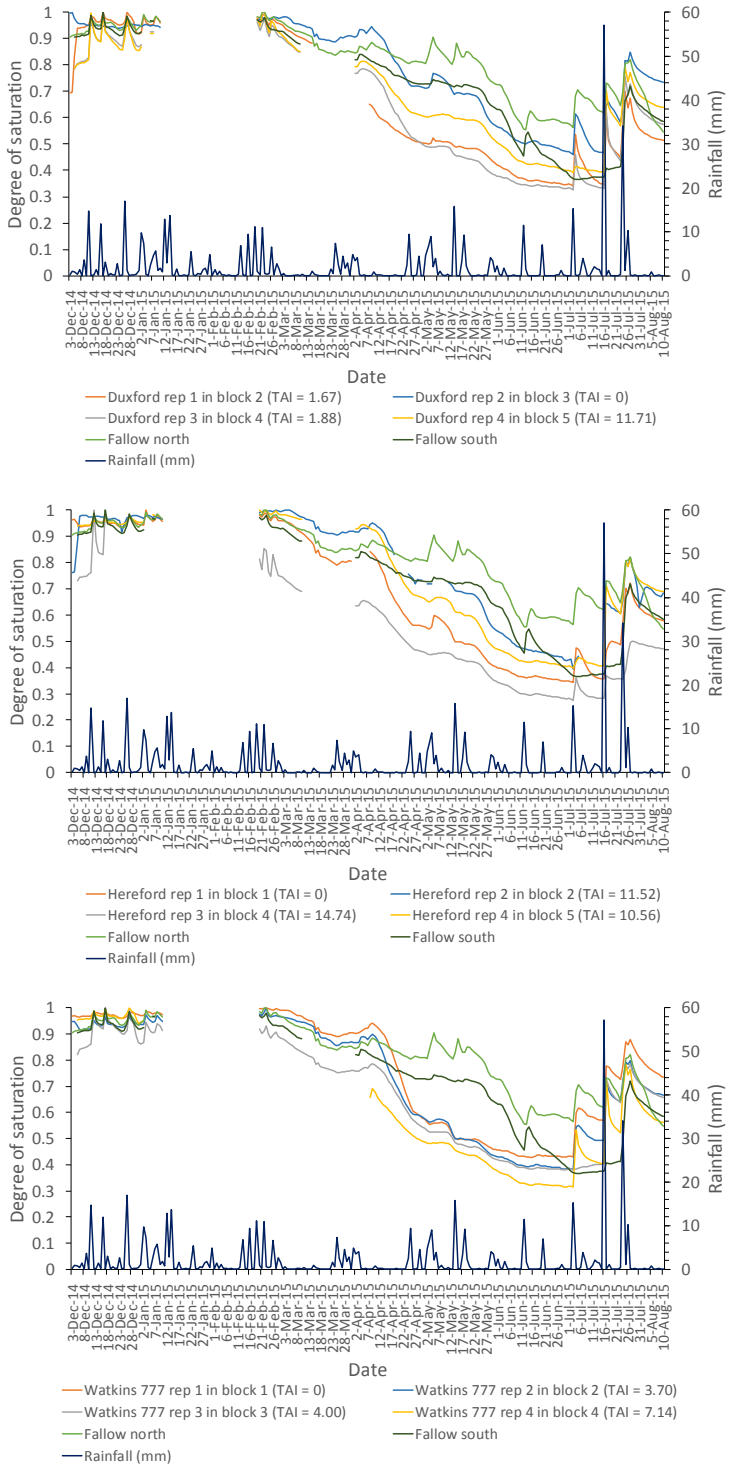



**Figure 3.16.** Degree of saturation from the four plot replicates of the moderately resistant varieties and two fallow plot replicates for variety Duxford (top), variety Hereford (middle) and Watkins line 777 (bottom) for the 2014-2015 field trial plotted against daily rainfall (mm) from 03/12/14 - 10/08/15. Field trial experimental block number and take-all index (TAI) indicated next to each plot replicate.

The degree of saturation of each plot replicate was then plotted against the TAI for the individual plot replicates for each variety (Figure 3.17 & Figure 3.18). The graphs for the take-all susceptible varietes were plotted on the same scale on the x axis as the 2013-2014 data, in order to easily compare between the two field seasons (Figure 3.17). However, the x axis scale was altered to accommodate for the low TAI scores for the moderately resistant varieties (Figure 3.18). Overall, the 2014-2015 field trial had a lower level of take-all pressure than seen in the 2013-2014 field trial (Table 3.5). The ranking of the TAI score varied across the blocks for each variety (Figure 3.17 & Figure 3.18). This made it difficult when looking at the relationship between the soil moisture data and the TAI. There appeared to be no relationship between the degree of saturation and TAI of the moderately resistant varieties of Duxford, Hereford and Watkins line 777, due to the extremely low TAI scores across the varieties (Figure 3.18). There was more of a spread in the TAI across the four plot replicates of the take-all susceptible varieties of Hereward and Paragon (Figure 3.17). However, there appeared to be no relationship between the degree of saturation and the TAI across the plot replicates for either variety. The plot replicate of Hereward in block 4 had the highest TAI across the Hereward plot replicates, yet the degree of saturation was intermediate across the five dates when compared to the other plot replicates (Figure 3.17). The plot replicate of Paragon in block 3 had the highest TAI across the Paragon plot replicates and a marginally higher degree of saturation than the other plot replicates on the 28$^{th}$ May, 25$^{th}$ June and 10$^{th}$ July. However, the replicate had the lowest degree of saturation by the end of the season on the 9$^{th}$ August.

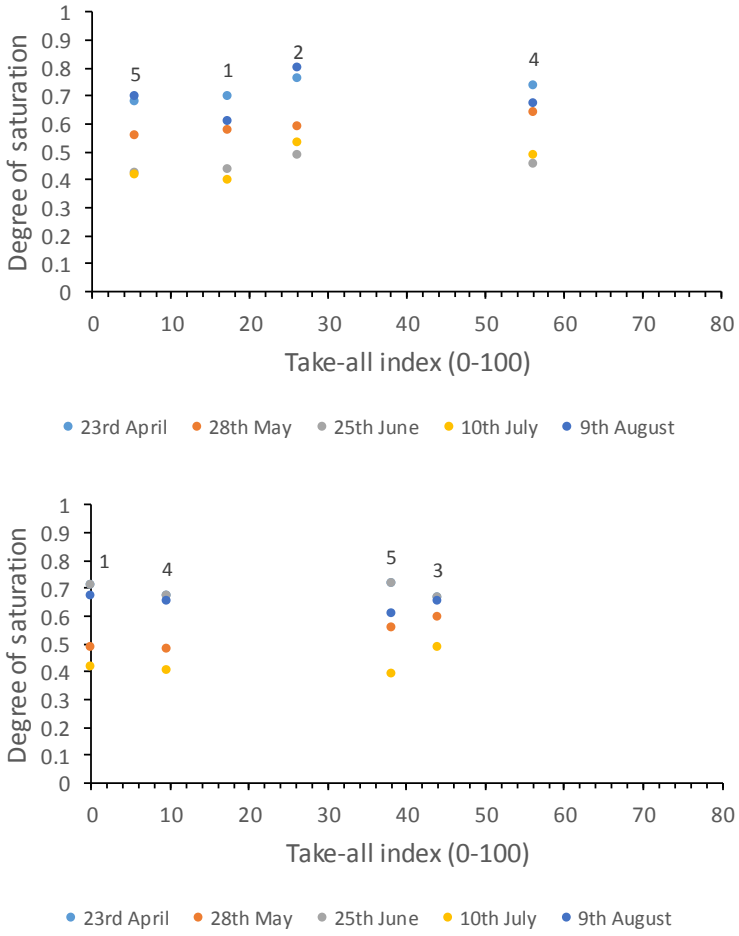



**Figure 3.17.** Degree of saturation for four plot replicates (experimental blocks numbered above) of the take-all susceptible varieties Hereward (top) and Paragon (bottom) plotted against the take-all index (TAI) for 23rd April, 28th May, 25th June, 10th July and 9th August of the 2014-2015 (2015/R/WW/1510) field trial.

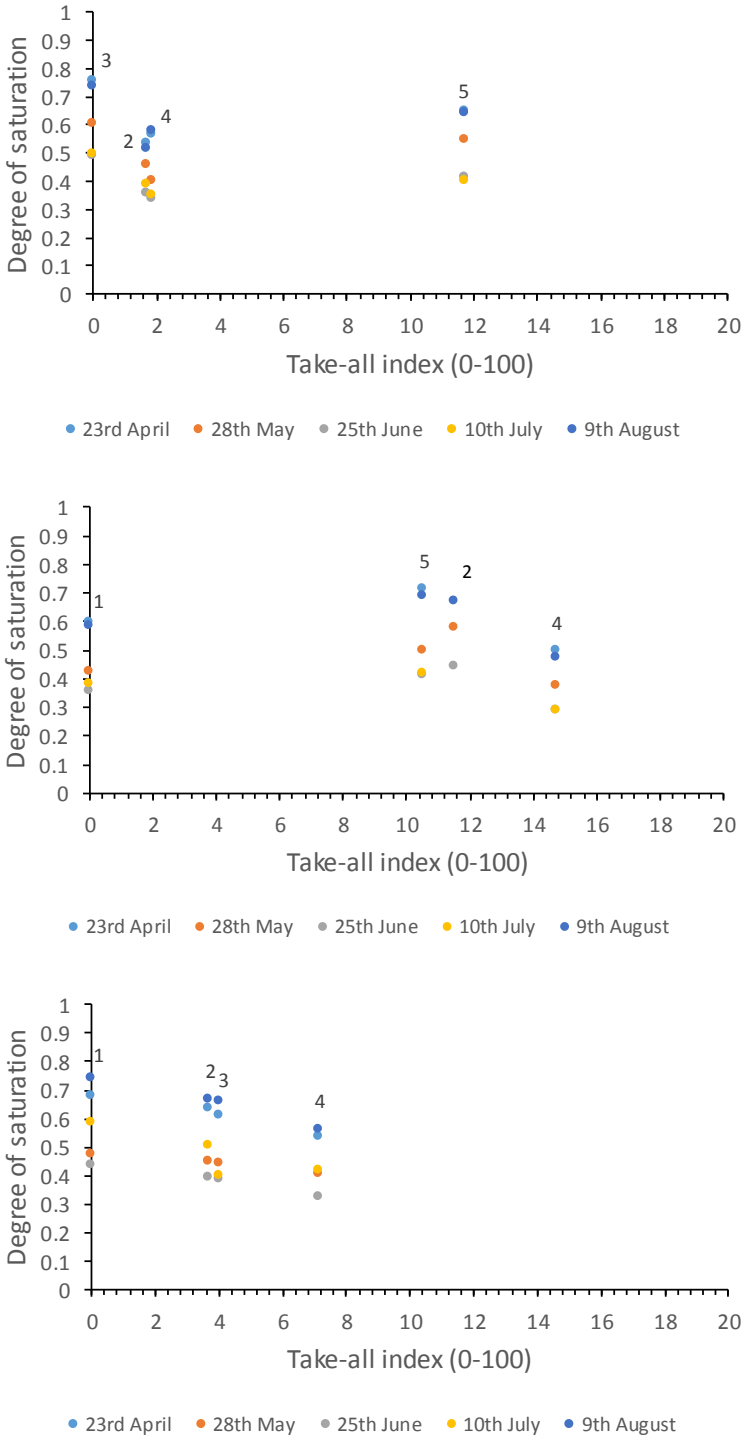



**Figure 3.18.** Degree of saturation for three or four plot replicates (experimental blocks numbered above) of the take-all moderately resistant varieties Duxford (top), Hereford (middle) and Watkins line 777 (bottom) plotted against the take-all index (TAI) for 23rd April, 28th May, 25th June, 10th July and 9th August of the 2014-2015 (2015/R/WW/1510) field trial. Note a change in the x axis scale.

As seen with the 2013-2014 field trial data, the relationship between the TAI and the degree of saturation was further explored by plotting the degree of saturation after a major rainfall event to look at the difference in the level of soil drying across the varieties for the 2014-2015 field trial data. There was an extreme rainfall event on 16$^{th}$ July 2015 where a total daily rainfall of 57.1 mm fell (see Figure 3.13). The next six consecutive days were plotted against the TAI for each individual plot (Figure 3.19 & Figure 3.20). One plot replicate of the take-all susceptible variety Hereward and one plot replicate of the take-all susceptible variety Paragon as well as two plot replicates of the moderately resistant variety Hereford, illustrated very little or no soil drying, an opposite trend to the remaining plot replicates of the five varieties (Figure 3.19 & Figure 3.20). The majority of the remaining plot replicates of the five varieties, illustrated a substantial drop in the degree of saturation from the 17$^{th}$ July to the 18$^{th}$ July followed by slight soil drying (Figure 3.19 & Figure 3.20). Although the moderately resistant take-all varieties illustrated a level of take-all resistance in the 2014-2015 field trial, there did not appear to be a difference in the level of soil drying between the moderately resistant take-all varieties and the take-all susceptible varieties.

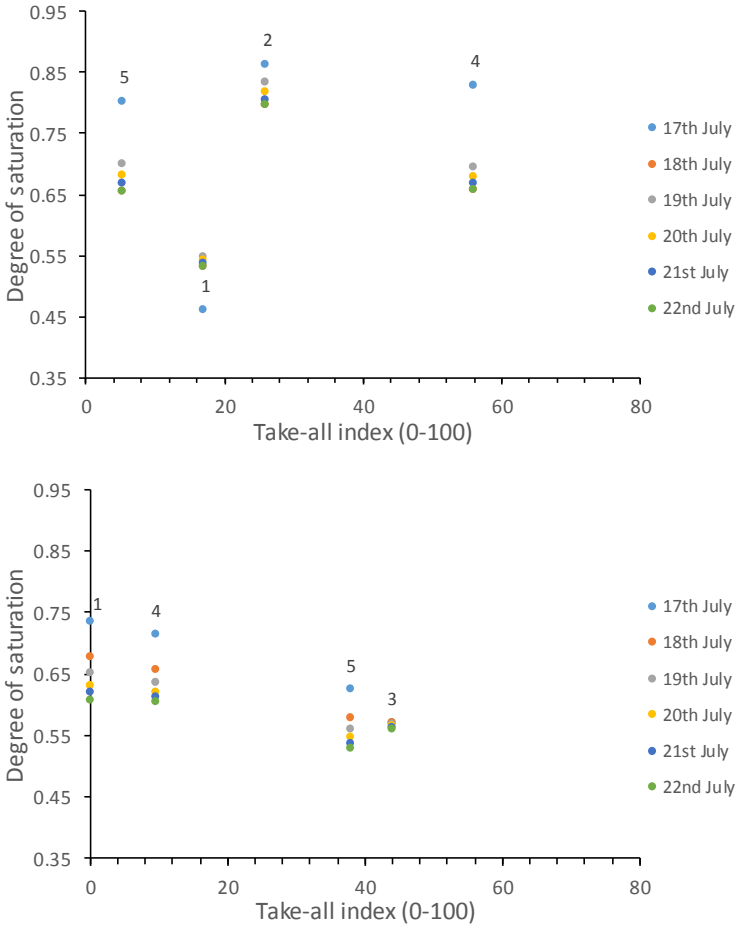



Figure 3.19. The level of soil drying after a major rainfall event on the 16th July 2015. The degree of saturation for the four plot replicates (experimental blocks numbered above) of the take-all susceptible varieties Hereward (top) and Paragon (bottom) plotted against the take-all index (TAI) for six consecutive days after the 16th July of the 2014-2015 (2015/R/WW/1510) field trial.

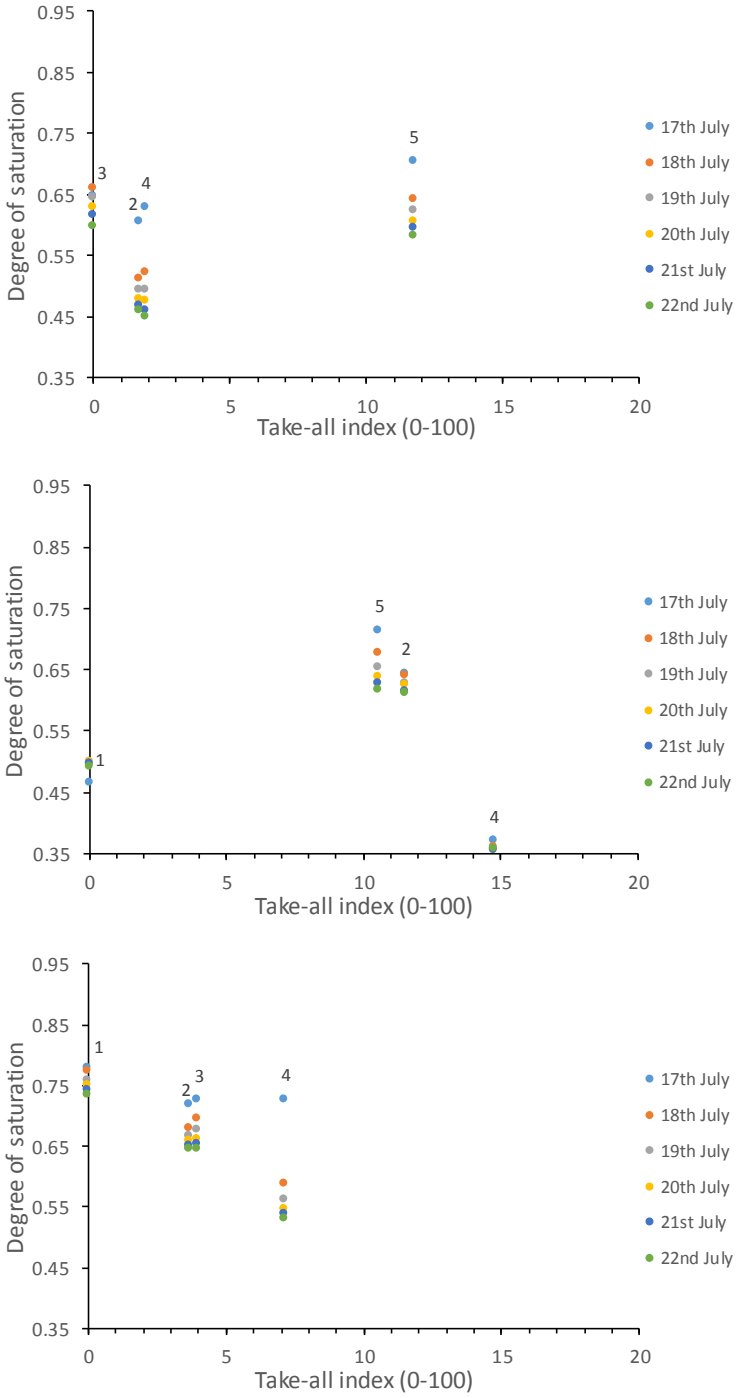



**Figure 3.20.** The level of soil drying after a major rainfall event on the 16th July 2015. The degree of saturation for the four plot replicates (experimental blocks numbered above) of the take-all moderately resistant varieties Duxford (top), Hereford (middle) and Watkins line 777 (bottom) plotted against the take-all index (TAI) for six consecutive days after the 16th July of the 2014-2015 (2015/R/WW/1510) field trial. Note a change in the x axis scale.

## 3.4 Discussion

### 3.4.1 Response of hexaploid wheat varieties to take-all disease in the field and their soil moisture performance

Two field trials were carried out to further characterise the 25 most take-all resistant varieties and to test the upper root function of two take-all susceptible varieties and three take-all moderately resistant varieties by measuring the soil water content through the use of *in situ* soil moisture sensors. The field trial in 2013-2014 was under a moderate disease pressure (TAI range = 6.86 - 59.61, grand mean = 37.17), whereas the 2014-2015 field trial was under a lower disease pressure (TAI range = 1.20 - 21.99, grand mean = 8.61). The two field trials revealed differences in the level of take-all across the screened varieties. The differences across the 33 screened varieties in 2013-2014 were statistically different, with a large spread in the TAI across the 25 most resistant varieties. The best performing Watkins varieties in the 2013-2014 field trial were 500 and 739. Although differences in the TAI were not statistically different across the five varieties that were assessed during the 2014-2015 field trial, there was a trend for the two take-all susceptible control varieties to have a higher TAI than the three varieties from the 25 most resistant take-all varieties.

Differences were found between the five varieties with inserted soil moisture sensors, take-all susceptible varieties Hereward and Paragon and take-all moderately resistant varieties Duxford, Hereford and Watkins 777, in their susceptibility to take-all infection across the two field seasons. During the moderate disease pressure of 2013-2014, the five varieties all had a moderate TAI score with little difference between the two take-all susceptible control varieties and the three varieties from the 25 most take-all resistant varieties. However, there was a trend for the two take-all susceptible control varieties to have a higher TAI during the 2014-2015 field trial. In particular, Hereward had a much higher TAI than the three take-all moderately resistant varieties. The 2014-2015 field trial was under a low take-all disease pressure and it may be that the resistance found in the three varieties, from the 25 most take-all resistant varieties, is more consistent in a low disease pressure year as seen in the 2014-2015 field season.

Above-ground phenotypic crop measurements were recorded across both the 2013-2014 and 2014-2015 field trials. Significant differences were found between the five wheat varieties across the two field trial years in the date that they reached anthesis, the date that they reached leaf senescence and the height of the plant. The 2014-2015 field trial was sown a week earlier than the 2013-2014 field trial, yet the varieties reached anthesis at a later date in 2015 (between 3-7 days) than found in 2014. Ear development and emergence are affected by weather conditions, cool and bright conditions leading up to flowering can prolong the ear formation stage (Wheat Growth Guide, AHDB Cereals and Oilseeds). The varieties reached leaf senescence at a slightly earlier date in 2015 (between 1-3 days) than 2014. Sizeable earliness in the senescence of the crop canopy is often associated with drought conditions or from disease (Wheat Growth Guide, AHDB Cereals and Oilseeds). The five varieties were generally a few centimetres taller in the 2013-2014 trial than the height recorded in 2014-2015 trial. The height of a wheat variety is largely dependent upon the variety itself, the sowing date as crops sown earlier may have an extra internode in addition to whether plant growth promoters (PGRs) have been applied since crops without the application of PGRs can be up to 20 cm taller (Wheat Growth Guide, AHDB Cereals and Oilseeds). The difference in the sowing date of the two field trials was marginal and PGRs were applied during both trials. Therefore, these factors would not have affected the very slight increase in the varietal plant height during the 2013-2014 field trial. The small difference in the plant height may have been affected by external environmental factors such as the weather conditions, nutrient availability or the difference in the soil type and soil properties. Significant correlations were found for the five varieties between the number of days to anthesis in the 2013-2014 trial and the number of days to anthesis in the 2014-2015 trial, as well as between the plant height in the 2013-2014 trial and the plant height recorded in the 2014-2015 trial. However, a large amount of variation was found in the ranking of the varieties across the two years for the date that the varieties reached total leaf senescence. This suggests that there are consistencies in the above-ground measurements of days to anthesis and plant height across the two field seasons. There were no significant relationships between the above-ground phenotypic crop measurements and take-all infection,

suggesting that it would be difficult to use above-ground measurements as a visual field scoring system for take-all disease in field trials.

The below-ground root measurements of number of seminal roots per plant, number of crown roots per plant and the total dry weight of the sampled root system were recorded across the two years along with the number of tillers per plant. Significant differences were found across the 33 varieties for all of the measurements in the 2013-2014 trial. The measurements were only recorded for the five varieties with the inserted soil moisture sensors during the 2014-2015 field trial. Significant differences were found across the five varieties for the number of seminal roots and the total dry weight of the sampled root system in 2015. A greater number of tillers and a greater number of crown roots and a subsequent greater total dry weight was found for the 2013-2014 field trial compared to the measurements in the 2014-2015 trial. The plant density of a crop can affect the number of crown roots and number of tillers per plant (Wheat Growth Guide, AHDB Cereals and Oilseeds). The greater the plant density, associated with seed rate and establishment of the crop, the fewer the number of crown roots and the fewer the number of tillers. The seed rate was 350 seeds per plot for both years and both field trials had a good crop establishment. The differences in the crown root counts may be due to the heterogeneity of the soil, leading to environmental noise in the below-ground measurements (Kuijken et al., 2015). The *T. monococcum* accessions had similar numbers of both seminal and crown roots as the Watkins, Gediflux and elite varieties, yet significantly more tillers, suggesting a different root-shoot architecture with more tillers being supported per root. This trait could potentially be beneficial in modern wheat breeding programmes since higher tiller counts can increase the yield potential. Yet, there is also the risk that a higher tiller count could lead to the detrimental effect of lodging. There was a strong negative relationship between the plant height and the total dry weights of the five varieties across both field trials, illustrating that the taller the plant the lighter the root system. This trend is opposite to what you would expect and caution should be taken due to the small sample size of only five varieties.

Of the below-ground root measurements, a relationship was only found between the seminal root counts and the TAI for 2013-2014 field trial. As expected, rye illustrated the highest crown root counts across the 34 varieties screened in the 2013-2014 field trial (Skou, 1975). Once the additional varieties from the 2014-2105 field trial are assessed, it will be interesting to see whether there was any evidence of re-rooting in rye in the 2013-2014 field trial in the presence of a moderate take-all disease pressure.

*In situ* soil moisture sensors revealed variability amongst varietal plot replicates in the soil water content across both field trials. There are a number of reasons that could account for the variation, including the heterogeneity nature of the soil which may range in soil density and include a number of air pockets. The stainless steel rods of the soil moisture sensors need to come into close contact with the surrounding soil in order to accurately measure the volumetric soil moisture content. The presence of soil air pockets surrounding the rods, may result in a reduced soil moisture content reading (ThetaProbe Soil Moisture Sensor Type ML2x User Manual). There is also an issue of soil air pockets when sensors need to be removed and subsequently re-inserted (ThetaProbe Soil Moisture Sensor Type ML2x User Manual), which was required for a few sensors during both field trials. There may also be slight undulations in the gradient across the two arable fields, resulting in increased drainage of specific areas of the field and therefore resulting in variations in the soil moisture content measurements across the field site.

The degree of saturation of the soil water content values was calculated to attempt to normalise the data, a reliable method shown by Whalley et al. (2004). In general, the fallow plots across both field seasons had a higher degree of saturation than the wheat varietal plots and this was as expected due to the lack of plant material to decrease the amount of water present in the top 15 cm soil layer (Whalley et al., 2008). Soil saturation was found during the winter months and from mid-March onwards the plots transitioned into a drying phase, this is comparable to Longobardi (2008), yet Whalley et al. (2008) found that the drying phase began slightly later from May. Due to the soil moisture sensors being only 15 cm below the surface, a high amount of evapotranspiration of the plots and a reduction in

rainfall results in drying of the soil (Longobardi, 2008). During the late spring to summer months, the soil became wetter in response to heavy rainfall events, especially during the 2015 summer.

As discussed previously, there was little difference in the level of take-all infection across the susceptible take-all varieties and the moderately resistant take-all varieties. The degree of saturation was plotted against the TAI of each plot and a positive linear relationship was found for three plot replicates of the highly susceptible take-all variety Hereward and three plot replicates of the moderately resistant take-all variety Watkins 777 in the 2013-2014 field trial. The higher the TAI of a plot, the higher the plots soil water content. This would correlate with the view that a higher level of take-all infection would lead to a greater amount of black necrotic lesions that would damage the roots and consequently disrupt the roots uptake of water, leading to an increase in surrounding soil moisture (Pillinger et al., 2005). The expectation would be that you would need a certain level of disease to negatively impact the roots and subsequently affect the soil moisture levels. However, the susceptible take-all variety Paragon illustrated similar TAI scores to Hereward, yet there was no relationship found with the degree of saturation of the individual plots. Similarly, the moderately resistant varieties Duxford and Hereford did not demonstrate a relationship between the two factors. Due to the low take-all disease pressure in the 2014-2015 field season, it was difficult to evaluate the relationship between the degree of saturation of the plot and the TAI, especially for the moderately resistant wheat varieties that, unlike in 2013-2014, illustrated strong resistance to take-all. There appeared to be no trends between soil moisture and take-all infection for the 2014-2015 date. Interestingly, as illustrated within this chapter, there was a high amount of rainfall during July in 2015 and the 2015-2016 season has seen a high level of take-all disease. Previous studies have found that take-all severity of a crop is affected by a high amount of rainfall in the previous spring and summer months (Roget and Rovira, 1991).

The relationship between take-all infection levels and the level of soil drying, following a major rainfall event, was examined for both field trials. Again, due to similar take-all infection levels across the susceptible and moderately resistant

varieties, it was difficult to evaluate the effect that take-all had on the level of soil drying in the 2013-2014 field trial. A difference in the level of soil drying between the moderately resistant take-all varieties and the take-all susceptible varieties was not found for the 2014-2015 trial. One of the main issues of studying take-all is the patchy nature of the disease. The analyses within this chapter have relied upon one soil moisture sensor per plot. Although the plots are small (1 m x 1 m), the level of take-all inoculum within a plot can vary and could have led to some of the obscurities in the data analysis.

### 3.4.2 Further work

The remaining take-all assessments from the 2014-2015 field trial will be assessed by Vanessa McMillan over the coming months as part of the WISP project, allowing a comparison of the TAI with the 2013-2014 field season results.

There has been a number of limitations within this chapter when evaluating the relationship between take-all infection and soil moisture. The main limitation was the fact that only one sample point at the end of the season was assessed for take-all levels of the field plots. In order to fully answer the working hypothesis that soil surrounding the most heavily take-all diseased plants would be the wettest, plant samples would need to be taken throughout the spring and summer months to allow a direct correlation with soil water content for individual dates. This would allow a more in depth analysis of the data to account for weather conditions on the build-up of take-all through the season and how this subsequently affected the soil moisture under individual plots. Unfortunately, due to small plot sizes (1m x 1m), plant samples could not be taken throughout the growing season and the analysis had to rely upon one sampling point at the end of the season to provide a TAI score of individual plots.

More recent work has focussed upon the development of dielectric sensors to be used in access tubes (Whalley et al., 2004). The use of access tubes is an easy solution to allow the sensors to be used at a range of increasing depth. The *in situ* dielectric soil moisture sensors used throughout this chapter were placed at a depth of 15 cm, since the relationship of the soil water content and the level of

take-all disease was being assessed and take-all inoculum is known to occupy the top 10-20cm of the soil (Cook, 2003). Take-all infection is known to reduce the rooting depth of a plant. It would be interesting to access the soil water profile at a range of depths under a high take-all disease pressure for both take-all susceptible and take-all resistant hexaploid wheat varieties. It would not have been possible to have investigated the soil water profile at depth within the PhD project since the access tubes could not be installed into the small plot sizes (1m x 1m) of the field trials.

### 3.4.3 Summary

This project has provided the first insight into the use of soil moisture sensors to evaluate the upper root performance of two susceptible and three moderately resistant varieties to take-all in two third wheat field trials. Although the three moderately resistant varieties indicated a high level of resistance in the 2014-2015 field season, the varieties lacked a level of resistance during the moderate take-all disease pressure season of 2013-2014. The study has not found a consistent relationship between the level of take-all infection and the level of soil moisture and preliminary results suggests that soil moisture sensors solely at a depth of 15 cm could not be used as a discriminatory tool for field screening wheat varieties for take-all infection.

# Chapter 4. Characterisation of the genetic basis of resistance in *Triticum monococcum*

## 4.1 Introduction

Partial resistance to take-all disease has previously been identified in a select number of *Triticum monococcum* accessions (Nilsson, 1969, McMillan et al., 2014), yet the genetic basis of this resistance has not been explored. One of the main aims of this PhD project was to screen a *T. monococcum* mapping population between the partially resistant accession MDR046 and the susceptible accession MDR037 under natural populations of the take-all fungus in the field. The mapping population has been sent for genotyping via microarray analysis using the Axiom® Wheat Breeder's Genotyping Array. An initial quantitative trait locus (QTL) analysis will then carried out to determine the genomic location of the genes responsible for the resistance to take-all and to provide the first indication of the complexity of the genetic basis of resistance to take-all in the parental line MDR046.

*Triticum monococcum* ($A^m$ $A^m$), also known as einkorn wheat, is a diploid wheat species that was domesticated from its wild progenitor *Triticum boeoticum* in the Karacadağ Mountains region of Turkey around 10,000 years ago (Heun et al., 1997). The domesticated species represented a hardy but low-yielding wheat crop and was widely cultivated during early farming practices in the Fertile Crescent (Feuillet et al., 2008). There are distinct morphological differences between domesticated *T. monococcum* and wild *T. boeticum*. *Triticum monococcum* produces larger seeds, has a tough rachis that holds the seed together which prevents the risk of shattering during harvesting and the ear breaks into individual spikelets upon pressure, allowing the wheat to be easily threshed compared to wild *T. boeticum* (Zohary and Hopf, 2000). Yet *T. monococcum* was abandoned before the Bronze Age and replaced with higher-yielding polyploid wheat species and has therefore been left to grow in its natural environment, devoid of further human selective pressure (Zohary and Hopf, 2000). *Triticum monococcum* is now considered a relic crop. *Triticum monococcum* is closely related to *Triticum urartu* (Ling et al., 2013),

with the latter the predominant A genome donor of some of the most significant polyploidy wheat species including the hexaploid bread wheat (*Triticum aestivum*) (Mayer et al., 2014) and durum wheat (*Triticum turgidum*) (Jing et al., 2007). The *T. monococcum* A genome (Fox et al., 2014), in contrast to *T. urartu* is underrepresented in modern wheat germplasm, only being used for the generation of *Triticum zhukovskyi* (Dvorak et al., 1993) and devoid of modern breeding bottlenecks (Kilian et al., 2009). This provides the opportunity to exploit and introduce potentially useful traits from *T. monococcum* for modern wheat genetic improvements.

*Triticum monococcum* accessions have been found to confer resistance to a number of plant pathogens (Table 4.1). Wild relatives provide an increase in genetic diversity that has the potential to be used in modern crop improvement programmes to address the narrow genepool of current elite wheat varieties. However it is difficult to cross *T. monococcum* to bread wheat (*T. aestivum*) and studies have found that direct crossing can results in embryo abortion and the failure of complete endosperm development (Bhagyalakshmi et al., 2008). The most efficient way of introgressing disease resistance traits from the species into current wheat varieties is to generate amphiploids between the diploid species and tetraploid species which are then crossed to a cultivated wheat species (Wulff and Moscou, 2014).

A number of *T. monococcum* accessions, conferring resistance to plant pathogens, have been crossed to hexaploid wheat (Table 4.1). Yet introgression of these *T. monococcum* disease resistance genes into hexaploid wheat has had varying success. It is important to assess the effect of the wild relative genes once introgressed into a hexaploid wheat background. The stem rust resistance gene *Sr22* has previously been introgressed from *T. monococcum* into the hexaploid variety Marquis (Kerber and Dyck, 1973) (Table 4.1). The *Sr22* introgression although conferring rust resistance, has however been associated with detrimental agronomic features such as a decline in yield (Paull et al., 1994). The and McIntosh (1975) also found that there was a reduced transmission of gametes carrying the resistance gene. These features have been associated to linkage drag from the

substantial amount of chromatin inherited from the *T. monococcum* species (Olson et al., 2010). Linkage drag is one of the main drawbacks of using wild relatives in crop breeding improvement strategies since eliminating the deleterious alleles is tedious and time-consuming (Feuillet et al., 2008). These genetically linked negative alleles have resulted in the lack of uptake of the *Sr22* gene worldwide (Olson et al., 2010). Yet, this particular stem rust resistance gene is still of great interest due to its resistance against the highly virulent stem rust race TTKSK (Ug99) (Jin et al., 2007), a new race that overcame the resistance gene *Sr31* that used to be present in 70% of elite wheat cultivars globally (Pretorius et al., 2000). Olson et al. (2010) have more recently identified *Triticum boeoticum* (syn. *T. monococcum* ssp. *aegilopoides*) recombinants with reduced amounts of chromatin that could potentially provide more useful material for deployment of the *Sr22* gene. The stem rust resistance gene *Sr21* (Table 4.1) had also been shown to confer resistance to the TTKSK race at the seedling stage (Pretorius et al. 2000) but the resistance appeared to become diluted when the resistance gene (The, 1973) was introgressed into hexaploid wheat (Jin et al., 2007). The stem rust resistance gene *Sr35*, also derived from *T. monococcum* (McIntosh et al., 1984) (Table 4.1), has been found to confer resistance against the TTKSK race when introgressed into hexaploid wheat (Saintenac et al., 2013). However, the *Sr35* gene has been found to be susceptible to a subset of stem rust races and therefore could not be singly deployed into hexaploid wheat (Saintenac et al., 2013). A gene conferring strong resistance to all tested stem rust races has been introgressed from the wild relative *Aegilops tauschii* into bread wheat, yet this gene only appears to confer moderate resistance to the TTKSK race (Periyannan et al., 2013). This highlights the need for multiple sources of resistance to allow successful control of plant pathogens. Pyramiding multiple resistance genes can increase the durability of resistance against a particular pathogen(s) (Saintenac et al., 2013).

Table 4.1. Examples of resistance in *Triticum monococcum* accessions to various plant pathogens.

| Plant pathogen | *Triticum monococcum* source | Resistance identified/gene | Chromosomal location | Introgressed into hexaploid wheat |
|---|---|---|---|---|
| *Blumeria graminis* f. sp. *tritici* | *T. m* accession NC96BGTA4 | Resistance identified (Srnić et al., 2005) | 7AL | Srnić et al. (2005) |
| | *T. m* accession NC96BGTA5 | Pm25 (Shi et al., 1998) | 1A | Shi et al. (1998) |
| | *T. m* accession TA2033 | *Mlm2033* (Yao et al., 2007) | *Xmag1759* - *Xmag2185* interval on 7A | |
| | *T. m* accession TA2026 | *Pm2026* (Xu et al., 2008) | $5A^{m}L$ | Xu et al. (2008) (tetraploid) |
| | *T. m* accession ATRI 1509/SLK | Resistance locus *Pm1b* (Hsam et al., 1998) | 7AL (*T. aestivum*) | Hsam et al. (1998) |
| | 70 *T. m* accessions | Resistance identified (Lebedeva and Peusha, 2006) | | |
| | *T. m* accession Tm27 | Resistance allele Pm4d (Schmolke et al., 2012) | 2AL | Schmolke et al. (2012) |
| Fusarium species causing head blight | 10-1 | Resistance identified (Fedak et al., 2007) | 5A linked to marker Xwme705 | Fedak et al. (2007) |
| | 7 *T. m* accessions | Resistance identified (Kopahnke et al., 2009) | 5A | |
| Karnal bunt | *T. m* accession | Resistance identified (Multani et al., 1988) | | Multani et al. (1988) |
| *Oculimacula acuformis* | *T. m* accessions | Resistance identified (Burt et al., 2010) | | |
| *Oculimacula yallundae* | *T. m* accessions | Resistance identified (Burt et al., 2010) | | |
| *Pseudocercosporella herpotrichoides* (syn. of *Oculimacula yallundae*) | 52 *T. m* accessions | Resistance identified (Cadle et al., 1997) | | |
| *Puccinia graminis* f. sp. *tritici* | *T. m* accession C.I.2433 | *Sr21* (The, 1973) | 2AL (The et al., 1979) | The (1973) |
| | *T. m* accession RL5244 | *Sr22* (Kerber and Dyck, 1973) | 7AL (The, 1973) | Kerber and Dyck (1973) |
| | *T. m* accession G2919 and accession C69.69 | *Sr35* (McIntosh et al., 1984) | 3AL | McIntosh et al. (1984) |
| *Puccinia recondita* f. sp. *tritici* | *T. m* accessions TmEinkorn 1,3 & Tm2126/5 | Resistance identified (Niks and Dekens, 1991) | | |
| | *T. m* accessions PI 266844 & PI 355520 | Resistance identified (Jacobs et al., 1996) | | Jacobs et al. (1996) |
| | *T. m accessions* | 3 resistance genes identified (Hussien et al., 1997) | 1A, 5A, 6A (*T. aestivum*) | Hussien et al. (1997) |
| | *T. m* accession TMR5-J14-12-24 | *Lr63* (Valkoun et al., 1988, McIntosh et al., 2009) | 3AS | Kolmer et al. (2010) |
| *Puccinia striiformis West. f.sp. tritici* | *T. m* accession pau 14087 | Resistance gene identified (Chhuneja et al., 2008) | *Xwmc407* - *Xwmc170* interval on 2A | Chhuneja et al. (2008) |
| *Puccinia triticina* | 152 *T. monococcum* accessions | Resistance identified (Anker and Niks, 2001) | | |
| Soil-borne cereal mosaic virus | *T. m* var. *macedonicum* accession K-38079 | Resistance identified (Kanyuka et al., 2004) | | |
| *Zymoseptoria tritici* | *T. m* accession MDR043 | *TmStb1* (Jing et al., 2008) | $7A^{m}$ | |

Partial resistance to take-all has been reported for a select number of *T. monococcum* accessions. Nilsson (1969) found that *T. monococcum* accessions varied considerably from those highly susceptible to take-all infection and others exhibiting a degree of resistance to the disease. More recently, since 2006, field trials have been carried out at Rothamsted Research, within the defra funded Wheat Genetic Improvement Network (WGIN; www.wgin.org.uk) programme, to evaluate the susceptibility of *T. monococcum* accessions to take-all infection. Data for field trials carried out in 2006, 2008, 2009, 2010 and 2011, as a third wheat crop, indicated a range of susceptibility between 34 *T. monococcum* accessions (Figure 4.1 shows data for 2008) (McMillan et al., 2014). Some accessions demonstrated susceptibility equivalent to that observed with the fully susceptible hexaploid cultivar Hereward. Others were found to be less infected, with disease levels equivalent or intermediate between the species control rye (highly resistant) and triticale (moderately resistant) (Figure 4.1) (McMillan et al., 2014). *Triticum monococcum* accessions MDR031 and MDR046 have consistently indicated a level of resistance intermediate between rye and triticale at both low and high disease pressures across different field sites and years on the Rothamsted Farm, Harpenden, UK (McMillan et al., 2014). These partially resistant accessions provide promising leads to explore the genetic basis of resistance to take-all with the potential of introgressing resistance genes into bread wheat as part of wheat breeding programmes (McMillan et al., 2014). The *T. monococcum* accessions MDR031 and MDR046 exhibit partial resistance to take-all and it is thought that the trait will be controlled by a number of genes or regions, unlike the *Triticum monococcum* - *Zymoseptoria tritici* interaction (Jing et al., 2008) (Table 4.1).

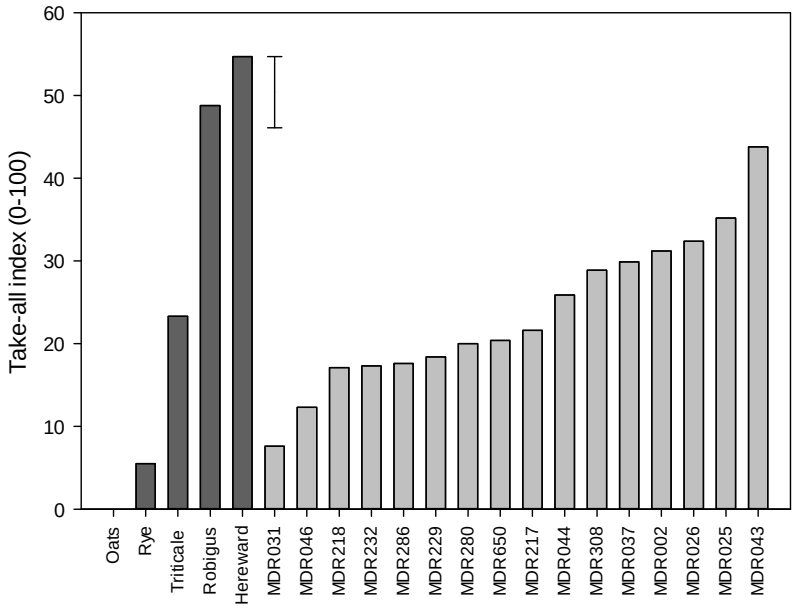



**Figure 4.1.** Susceptibility of *Triticum monococcum* accessions and controls to take-all disease in a third wheat field trial during the high disease pressure year of 2008 (ANOVA: $P = 0.002$, SED = 8.6). (McMillan et al., 2014).

The partially resistant *T. monococcum* accessions MDR031 and MDR046 have now been crossed to the fully susceptible accessions MDR037 and MDR043 and mapping populations have been subsequently developed by single seed descent (SSD). At the beginning of this PhD project, $F_5$:$F_6$ seed of the MDR046 (R) X MDR037 (S) population was available for field testing and this was selected to explore the genetic basis of take-all resistance in the *T. monococcum* MDR046 accession. This PhD study reports the first field screening of the MDR037 (S) X MDR046 (R) mapping population to take-all disease across two replicated field seasons. A pot bioassay was also carried out to screen the susceptibility of 34 *T. monococcum* accessions to take-all infection at the seedling stage. The majority of these accessions had previously been screened under third wheat field conditions, see Figure 4.1 and McMillan et al. (2014).

## 4.2 Materials and Methods

### 4.2.1 Screening of the *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population to take-all disease in the field

Two field trials were carried out to screen the *T. monococcum* MDR037 (S) X MDR046 (R) mapping population for resistance against take-all disease. The field trials were all established on the Rothamsted Farm (Table 4.2) and were carried out in a third wheat situation, to try to ensure a high natural take-all disease pressure. A randomised block design was calculated for each trial by statistician Rodger White (see Appendix 4.1 for field plans). *Triticum monococcum* is highly sensitive to herbicides and only one herbicide application (Pacifica®) was applied during the spring. The field plots were subsequently weeded by hand during the spring-summer months.

*Triticum monococcum* seed was prepared for each of the two field trials as described in Chapter 2. A number of MDR037 (S) X MDR046 (R) mapping population lines were multiplied in the glasshouse to provide seed for all field trials (detailed in Appendix 4.2). Five plants were grown of each line and seed was collected from these plants and stored in the cold room at 4°C until the seed was prepared for each field trial.

In 2014, 72 $F_{5:}F_6$ *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population lines were screened in the field along with the two *T. monococcum* parental lines and the hexaploid variety Hereward was used as a highly susceptible control to take-all (Table 4.2). The field trial consisted of five replicates of each of the *T. monococcum* lines, exceptions with between 2-4 replicates (for 14 lines) due to a lack of seed are detailed in Appendix 4.2. Take-all is a notoriously patchy disease and to enable the spatial variation of the disease to be accounted for during the statistical analysis, 25 plots of the highly susceptible control variety Hereward were sown throughout the field trial. To ensure a balanced block design of the field trial, a number of 'dummy' plots were included by statistician Rodger White. These 15 'dummy' plots were also sown with the field trial control variety Hereward to enable a greater number of plots to be included in the statistical analysis, a total of 40 Hereward plots (Appendix 4.1). During the months of January-March 2014, part

of the field trial was under water due to flooding. The number of germinated plants per plot was recorded to calculate the establishment of the field trial (see Appendix 4.3 and Figure 4.2). Twelve plots had no establishment and could not be sampled (Appendix 4.3). Twenty-four plots were very poorly established and had less than 6 plants (Appendix 4.3). Plots were weeded by hand on 09/05/14, 21/05/14 and 09/06/14.

In 2015, a repeat field trial was carried out. An additional seven *T. monococcum* MDR037 (S) X MDR046 (R) mapping population lines were bulked up in the glasshouse from the original seed and were included in the 2015 repeat field trial (Table 4.2). The field trial consisted of five replicates of each of the $F_5$:$F_6$/$F_5$:$F_7$ *T. monococcum* lines, except for only two replicates for lines 26 and 33 due to seed availability (Appendix 4.2). Again, 25 plots of the field trial highly susceptible control variety Hereward were sown along with the 16 'dummy' plots sown with Hereward, a total of 41 Hereward plots (Appendix 4.1). Plots were weeded by hand on 26/05/15. There were also significant wheat volunteers throughout the field trial and these were removed by hand on 12/05/15, 26/05/15, 04/06/15 and 05/06/15.



Figure 4.2. Photograph illustrating the effects of flooding on the establishment of 2013-2014 field trial (2014/R/WW/1418) with poor germination of plants and empty plots found in blocks 3 and 4 (photograph taken on 23/05/14).



Figure 4.3. Photograph of the 2013-2014 field trial (2014/R/WW/1418) two days before plant samples were taken (photograph taken on 14/07/14).

Sampling methods and measurements taken over the two field seasons are detailed in Table 4.3. Ear emergence/days to anthesis, flag leaf senescence/days to senescence and plant height were measured and recorded for each plot as detailed in Chapter 2. Plant samples were taken in the summer for each plot (see Figure 4.3), washed, dried and assessed for take-all infection as described in Chapter 2.

A rodent infestation during storage of the plant root systems in 2015 led to damage of the labels and the loss of seven plots from the analysis of the 2014/R/WW/1418 field trial (Appendix 4.3). Field trial data was analysed by statistician Rodger White. A Residual Maximum Likelihood (REML) analysis was used to statistically analyse the field trial data for 2013-2014 (field trial: 2014/R/WW/1418) because there was not enough seed for each treatment to be equally repeated within the blocks of the experiment and the experimental design was therefore unbalanced. The above-ground phenotypic crop measurements of plant height, ear emergence and leaf senescence were all statically analysed through REML. The take-all index (TAI) data

was logit transformed before performing a REML analysis. The REML analyses, of the 2013-2014 field trial data, were also adjusted to exclude plots with less than six plants for the TAI and take-all infection analyses of field trial 2014/R/WW/1418; these plots were referred to as missing in the model and accounted for the poor establishment from the flooding event (see Appendix 4.3).

A REML analysis was also used to statistically analyse the logit transformed TAI data in the repeat field trial in 2014-2015 (2015/R/WW/1512). An analysis of variance (ANOVA) analysis was used to statistically analyse the plant height, ear emergence and leaf senescence data from the 2014-2015 trial. Spearman's rank correlation was used to test the association between the above-ground measurements and the TAI. Spearman's rank correlation was also used to test the association of these measurements between the two field trials.

A cross-season REML analysis was used to analyse the combined TAI data from the 2013-2014 and 2014-2015 field trials. The TAI was transformed using the logit transformation.

Table 4.2. Experimental field trial design for the two *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population field trials.

| Field trial | Rothamsted field and soil type | Previous crop and variety | Parental and control lines | Number of screened *Tm* MDR037 X MDR046 lines | Plot size | Seed rate per plot | Sowing date |
|---|---|---|---|---|---|---|---|
| **2014/R/WW/1418** | Long Hoos 5 Batcombe - Carstens with sandier inclusions | Winter wheat Conqueror | MDR037 MDR046 Hereward | 72 | 4 rows x 0.6m length | 80 | 15/11/2013 |
| **2015/R/WW/1512** | Bylands Typical Batcombe | Winter wheat Conqueror | MDR037 MDR046 Hereward | 79 | 4 rows x 0.6m length | 80 | 10/10/2014 |

Table 4.3. Sampling and measurement details for the *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population field trials.

| Field trial | Sampling method | Units per plot | Date measured/sampled | Growth stage (GS) |
|---|---|---|---|---|
| **2014/R/WW/1418** | Ear emergence | GS | 13/06/14 - 08/07/14 | 37-61 |
| | Leaf senescence | 0-10 | 14-29/07/14 | 61-91 |
| | Plant heights | cm | 01/07/14 & 04/07/14 | 71 |
| | Plant samples | 3 x 20cm | 16-17/07/14 | 85-87 |
| **2015/R/WW/1512** | Ear emergence | GS | 02-26/06/15 | 45-61 |
| | Leaf senescence | 0-10 | 13-23/07/15 | 77-92 |
| | Plant heights | cm | 14/07/2015 | 77-83 |
| | Plant samples | 3 x 20cm | 22-23/07/15 | 87 |

### 4.2.2 DNA extraction of *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population

Carlos Bayon performed the DNA extractions on the *T. monococcum* MDR046 X MDR037 mapping population prior to sending for genotyping. Five seeds for each of the 79 *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population lines, along with the two parental accessions, were dehusked. The seeds were then grown in the glasshouse for ~ 2 weeks before two 4-6 cm sections of leaf tissue were harvested from one plant and each placed into a 2 ml microcentrifuge tube, frozen in liquid nitrogen and stored at -80°C. One of the tubes was stored as a back-up and DNA extractions were performed on the other tube. Ball bearings were added to the tube and samples were ground in a genogrinder at 1000 Hz for 3 minutes. DNA was extracted following a wheat DNA extraction protocol using 600 µl of extraction buffer (0.1 m Tris-HCl (pH 7.5) + 0.05 m EDTA (pH 8.0) and 1.25% SDS). Samples were then vortexed and spun briefly before incubating at 65°C for 1 hour with occasional mixing by inversion. The samples were moved to room temperature and 300 µl of 6M ammonium acetate was added and samples were vortexed and incubated on ice for 15-30 minutes. Samples were then centrifuged at 12,000 *g* for 10 minutes and the supernatant was transferred into a fresh tube before adding 550 µl of isopropanol and mixing the samples by inversion and incubating on ice for 5 minutes. The samples were centrifuged at 12,000 *g* for 10 minutes to pellet the DNA. The supernatant was removed and the tubes were inverted onto tissue to drain the residues. The pellet was then washed by adding 750 µl of 70% ethanol and the pellet was dislodged from the bottom of the tube before centrifuging at 12,000 *g* for 5 minutes to collect the DNA pellet. The supernatant was removed and the tube inverted onto tissue to drain the residues before allowing the pellet to air-dry for 30 mintutes. The pellet was resuspended in 50 µl reduced EDTA TE buffer (10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA (pH 8.0)). The extracted DNA was then quantified using the Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA) and the Qubit (Thermo Fisher Scientific, USA) and stored at -20°C. A 1% agarose gel was run at 90 volts for 30 minutes to visualise DNA degradation.

Extracted DNA was sent to Bristol Genomics Facility at the University of Bristol to be genotyped via microarray analysis using the Axiom® Wheat Breeder's Genotyping Array. The 79 *T. monococcum* MDR037 (S) X MDR046 (R) mapping population lines were prepared for a 384 format assay along with additional *T. monococcum* accessions that were to be genotyped as part of my supervisor Vanessa McMillan's work and were not part of this PhD project. DNA was diluted to a concentration of 30 ng / µl using reduced EDTA TE buffer (10 mM Tris - HCl (pH 8.0), 0.1 mM EDTA (pH 8.0)) and loaded into 4 x 96 well plates.

## 4.3 Results

### 4.3.1 Performance of *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population to take-all disease in the field

The monthly weather data for the two field seasons was downloaded from the Rothamsted electronic archive (e-RA) along with the departure from the 30 year means (1981-2010) (Table 4.4). The maximum and minimum mean temperatures for the 2013-2014 and 2014-2015 field trial seasons were similar to the 30 year means. The 2013-2014 season saw a milder winter and a warmer summer than the 2014-2015 season. The monthly rainfall for March, April, May and June were similar across the two years. In 2014, over twice as much rain fell in January than during the same month in 2015 and compared to the 30 year average, resulting in flooding of the 2013-2014 field trial and poor plant establishment on the right-hand side of blocks 3 and 4 (Appendix 4.3) (Figure 4.2, blocks 3 and 4 lie to the front of the photograph).

Table 4.4. Monthly weather summaries during 2013-2014 (2014/R/WW/1419) and 2014-2015 (2015/R/WW/1510) field trial seasons downloaded from e-RA.

| Monthly weather summary (Departure from the 30 year means (1981-2010) in brackets) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Mean temperature (°C) | | | | | |
| | | | Maximum | | Minimum | | Rainfall | |
| **Field season** | **Year** | **Month** | **(°C)** | **()** | **(°C)** | **()** | **Total (mm)** | **()** |
| **2014/R/WW/1418** | 2013 | November | 8.9 | (-0.85) | 3.6 | (-0.24) | 59.5 | (-17.13) |
| | 2013 | December | 9.1 | (+2.22) | 2.6 | (+0.92) | 123.1 | (+53.60) |
| | 2014 | January | 8.5 | (+1.83) | 2.9 | (+1.66) | 176.2 | (+106.25) |
| | 2014 | February | 9.0 | (+2.07) | 3.2 | (+2.31) | 141.9 | (+91.80) |
| | 2014 | March | 12.7 | (+2.84) | 3.4 | (+0.73) | 28.2 | (-22.57) |
| | 2014 | April | 14.8 | (+2.19) | 6.1 | (+2.12) | 31.5 | (-23.54) |
| | 2014 | May | 16.5 | (+0.41) | 8.3 | (+1.39) | 82.8 | (+28.14) |
| | 2014 | June | 20.1 | (+1.01) | 10.6 | (+0.88) | 30.5 | (-22.77) |
| | 2014 | July | 23.9 | (+2.11) | 13.3 | (+1.40) | 36.9 | (-13.00) |
| **2015/R/WW/1512** | 2014 | December | 8.1 | (+1.20) | 2.1 | (+0.41) | 64.0 | (-5.57) |
| | 2015 | January | 7.5 | (+0.76) | 0.9 | (-0.32) | 81.9 | (+11.93) |
| | 2015 | February | 6.7 | (-0.21) | 0.8 | (-0.17) | 54.6 | (+4.42) |
| | 2015 | March | 10.2 | (+0.27) | 2.9 | (+0.27) | 26.1 | (-24.67) |
| | 2015 | April | 14.5 | (+1.85) | 4.0 | (-0.04) | 31.0 | (-24.05) |
| | 2015 | May | 15.5 | (-0.53) | 7.0 | (+0.12) | 68.4 | (+13.75) |
| | 2015 | June | 19.5 | (+0.33) | 9.2 | (-0.58) | 26.7 | (-26.55) |
| | 2015 | July | 21.4 | (-0.40) | 11.7 | (-0.15) | 132.6 | (+82.71) |

#### 4.3.1.1 Take-all susceptibility of the 2013-2014 field trial experiment

The 2013-2014 field trial screened 72 MD0R37 (S) X MDR046 (R) mapping population lines and there was no significant difference of control varieties or of mapping population lines for the TAI score ($P$ = 0.064) (Table 4.5). A trend for a higher level of infection in the susceptible parent is however apparent and a range of take-all infections exist within the population, with take-all indexes ranging from 9.52-58.85 (Table 4.5). The *T. monococcum* partially resistant parental line MDR046 had a TAI of 25.54 and the susceptible parental line MDR037 a TAI of 42.55. The highly susceptible control Hereward had a lower than expected TAI of 38.29. The TAI across the control species indicates that a moderate level of disease was present across the field trial. *Triticum monococcum* MDR046 (R) X MDR037 (S) line 44 was the least susceptible line to take-all disease (TAI = 9.52). The TAI score per plot was also plotted against the establishment of the field trial (Figure 4.4). No trend was found for the level of plant establishment of the plot on the TAI score (Figure 4.4). The Hereward plots did however appear to have a better plant establishment in the flooded area of the field trial than the *T. monococcum* plots (Appendix 4.3) and there did not appear to be a visual difference in the structure of Hereward adult root systems between a flooded plot and a non-flooded plot (Figure 4.5 & Figure 4.6).

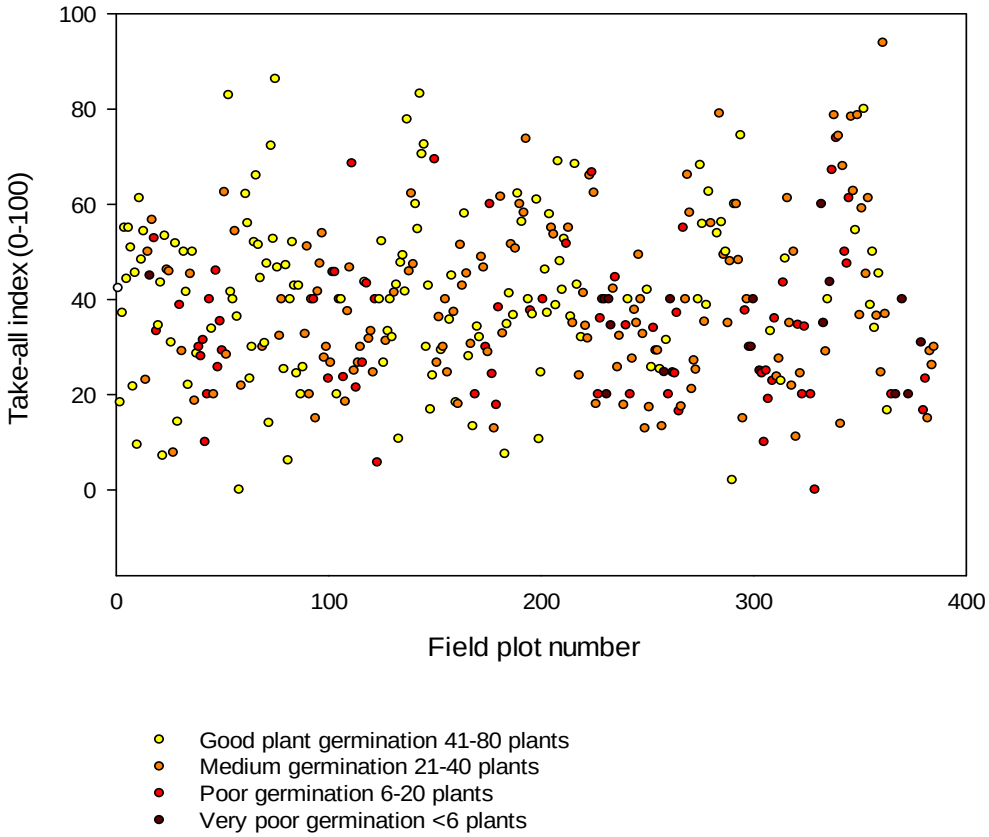



Figure 4.4. Take-all index score (0-100) of *Triticum monococcum* and Hereward lines plotted against the establishment of their field trial plot for 2013-2014 field trial (2014/R/WW/1418).



**Figure 4.5.** Photograph of adult plant root systems of the susceptible control variety Hereward, plot 304 of the flooded block 4, of the 2013-2014 field trial (2015/R/WW/1418). (Photograph credit: Graham Shephard of the Visual Communications Unit at Rothamsted Research).



**Figure 4.6.** Photograph of adult plant root systems of the susceptible control variety Hereward, plot 53 of the non-flooded left-hand side of block 3, of the 2013-2014 field trial (2015/R/WW/1418). (Photograph credit: Graham Shephard of the Visual Communications Unit at Rothamsted Research).

#### 4.3.1.2 Take-all susceptibility of the 2014-2015 field trial experiment

The 2014-2015 field trial screened an additional seven MD0R37 (S) X MDR046 (R) mapping population lines and significant differences were detected for the control varieties and mapping population lines in the TAI ($P$ = 0.001) (Table 4.5). The take-all indexes for the *T. monococcum* mapping population lines had a very similar range when compared to the 2013-2014 field trial (2014-2015: TAI range 8.91 - 50.84; 2013-2014: TAI range 9.52 - 58.85) (Table 4.5). Yet, a greater difference in the TAI, compared to the 2013-2014 data, was found between the two parental lines with a TAI of 13.08 for the partially resistant parental line MDR046 (Figure 4.7) and a TAI of 40.62 for the susceptible parental line MDR037 (Figure 4.8). The highly susceptible control variety Hereward had a TAI intermediate of the two parental lines of 25.78. The TAI across the control species indicates that a low to moderate level of disease was present across the field trial. This is also highlighted by the TAI grand mean for the trial of only 25.27 compared to a TAI grand mean of 37.78 in the 2013-2014 field trial. The TAI grand means for each field trial were also found to be equivalent to the TAI score of Hereward (Table 4.5). *Triticum monococcum* MD0R37 (S) X MDR046 (R) line 3 was the least susceptible line to take-all disease (TAI = 8.91), whereas the line indicated a moderate level of susceptibility to take-all disease in the 2013-2014 field trial (TAI = 39.25).



**Figure 4.7.** Photograph of adult plant root systems of the resistant *Triticum monococcum* parental line MDR046, plot 43, of the 2014-2015 field trial (2015/R/WW/1512). (Photograph credit: Graham Shephard of the Visual Communications Unit at Rothamsted Research).



**Figure 4.8.** Photograph of adult plant root systems of the susceptible *Triticum monococcum* parental line MDR037, plot 35, of the 2014-2015 field trial (2015/R/WW/1512). (Photograph credit: Graham Shephard of the Visual Communications Unit at Rothamsted Research).

Table 4.5. Susceptibility of the *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population lines and control varieties to take-all disease in the 2013-2014 (2014/R/WW/1418) and 2014-2015 (2015/R/WW/1512) field trials.

| | **Logit of take-all index (0-100), (back transformed means)** | | | |
|---|---|---|---|---|
| **Treatment** | **2014** | | **2015** | |
| **Controls** | | | | |
| MDR037 | -0.140 | (42.55) | -0.180 | (40.62) |
| MDR046 | -0.522 | (25.54) | -0.928 | (13.08) |
| Hereward | -0.228 | (38.29) | -0.516 | (25.78) |
| ***T. m* MDR037 X MDR046 mapping population lines** | | | | |
| Line 1 | -0.632 | (21.53) | -0.639 | (21.27) |
| Line 2 | -0.230 | (38.19) | -0.871 | (14.31) |
| Line 3 | -0.208 | (39.25) | -1.132 | (8.91) |
| Line 4 | 0.021 | (50.57) | -0.455 | (28.31) |
| Line 5 | -0.115 | (43.79) | -0.617 | (22.23) |
| Line 6 | -0.432 | (29.17) | -0.853 | (14.93) |
| Line 7 | -0.250 | (37.26) | -0.265 | (36.89) |
| Line 9 | -0.189 | (40.16) | -0.706 | (19.14) |
| Line 10 | -0.308 | (34.59) | -0.918 | (13.25) |
| Line 11 | | | -0.281 | (35.93) |
| Line 12 | -0.195 | (39.87) | -0.691 | (19.68) |
| Line 13 | | | -0.399 | (30.65) |
| Line 14 | -0.408 | (30.18) | -0.811 | (15.88) |
| Line 15 | 0.097 | (54.35) | -0.297 | (35.21) |
| Line 16 | -0.433 | (29.13) | -0.609 | (22.33) |
| Line 17 | -0.122 | (43.45) | -0.515 | (25.83) |
| Line 19 | -0.452 | (28.33) | -0.757 | (17.63) |
| Line 20 | -0.579 | (23.42) | -0.029 | (48.29) |
| Line 21 | -0.300 | (34.92) | -0.587 | (23.32) |
| Line 22 | -0.287 | (35.54) | -0.557 | (24.25) |
| Line 23 | -0.258 | (36.87) | -0.762 | (17.58) |
| Line 24 | -0.359 | (32.30) | -0.197 | (39.95) |
| Line 25 | -0.197 | (39.76) | -0.506 | (26.13) |
| Line 26 | -0.272 | (36.23) | -0.817 | (15.84) |
| Line 27 | -0.523 | (25.49) | -0.427 | (29.33) |
| Line 28 | -0.218 | (38.76) | -0.574 | (23.52) |
| Line 29 | 0.116 | (55.25) | -0.345 | (32.75) |
| Line 30 | -0.299 | (34.98) | -0.666 | (20.48) |
| Line 31 | -0.447 | (28.52) | -0.524 | (25.43) |
| Line 33 | -0.066 | (46.20) | -0.197 | (40.14) |
| Line 34 | -0.317 | (34.18) | -0.638 | (21.65) |
| Line 35 | 0.189 | (58.85) | -0.120 | (43.54) |

Table 4.5. Continued.

| | Logit of take-all index (0-100), (back transformed means) | | | |
|---|---|---|---|---|
| **Treatment** | **2014** | | **2015** | |
| ***T. m* MDR037 X MDR046 mapping population lines** | | | | |
| Line 36 | -0.194 | (39.94) | -0.268 | (36.40) |
| Line 37 | -0.102 | (44.42) | -0.513 | (25.91) |
| Line 38 | -0.594 | (22.85) | -0.286 | (35.51) |
| Line 39 | -0.283 | (35.72) | -0.533 | (25.05) |
| Line 40 | 0.160 | (57.41) | -0.291 | (35.45) |
| Line 41 | -0.191 | (40.09) | -0.818 | (15.76) |
| Line 42 | | | -0.301 | (35.14) |
| Line 43 | -0.435 | (29.04) | -0.657 | (20.53) |
| Line 44 | -1.098 | (9.52) | -0.403 | (30.40) |
| Line 45 | 0.013 | (50.16) | -0.595 | (22.85) |
| Line 46 | -0.448 | (28.50) | -0.528 | (25.40) |
| Line 47 | -0.198 | (39.72) | -0.391 | (30.67) |
| Line 48 | -0.222 | (38.57) | -1.070 | (10.01) |
| Line 50 | -0.287 | (35.53) | -0.674 | (20.19) |
| Line 51 | -0.608 | (22.36) | -0.874 | (14.37) |
| Line 52 | | | -0.308 | (34.40) |
| Line 53 | -0.242 | (37.64) | -0.393 | (30.65) |
| Line 54 | -0.028 | (48.12) | 0.023 | (50.84) |
| Line 55 | -0.301 | (34.88) | -0.672 | (20.37) |
| Line 56 | -0.371 | (31.77) | -0.474 | (27.84) |
| Line 57 | -0.205 | (39.42) | -0.522 | (25.50) |
| Line 58 | -0.321 | (33.99) | -0.556 | (24.20) |
| Line 59 | -0.190 | (40.11) | -0.507 | (26.05) |
| Line 60 | 0.096 | (54.27) | -0.734 | (18.34) |
| Line 61 | -0.153 | (41.91) | -0.601 | (22.58) |
| Line 62 | -0.280 | (35.85) | -0.468 | (27.68) |
| Line 63 | -0.302 | (34.83) | -0.789 | (16.61) |
| Line 64 | -0.266 | (36.50) | -0.763 | (17.20) |
| Line 65 | -0.114 | (43.83) | -0.538 | (25.13) |
| Line 66 | -0.165 | (41.35) | -0.626 | (21.77) |
| Line 67 | -0.171 | (41.04) | -0.857 | (14.67) |
| Line 68 | -0.193 | (39.98) | -0.935 | (12.84) |
| Line 69 | -0.487 | (26.92) | -0.749 | (17.72) |
| Line 70 | -0.152 | (41.94) | -0.484 | (26.83) |
| Line 71 | -0.209 | (39.18) | -0.712 | (19.09) |
| Line 72 | -0.242 | (37.61) | -0.764 | (17.35) |
| Line 73 | 0.031 | (51.03) | -0.560 | (24.15) |
| Line 74 | -0.381 | (31.32) | -0.563 | (23.90) |
| Line 75 | -0.055 | (46.75) | -0.433 | (29.05) |

Table 4.5. Continued.

| | Logit of take-all index (0-100), (back transformed means) | | | |
|---|---|---|---|---|
| **Treatment** | **2014** | | **2015** | |
| ***T. m* MDR037 X MDR046 mapping population lines** | | | | |
| Line 76 | -0.082 | (45.39) | -0.567 | (23.71) |
| Line 77 | | | -0.404 | (30.35) |
| Line 78 | | | -0.496 | (26.45) |
| Line 79 | -0.382 | (31.26) | -0.642 | (21.02) |
| Line 80 | -0.189 | (40.17) | -0.461 | (28.17) |
| Line 81 | -0.130 | (43.05) | -0.272 | (36.27) |
| Line 82 | -0.152 | (41.96) | -0.487 | (26.83) |
| Line 83 | | | -0.490 | (26.69) |
| d.f. | 74 | | 81 | |
| SED | 0.253 | | 0.250 | |
| F statistic | 1.32 | | 1.68 | |
| F probabilty | 0.064 | | 0.001 | |
| Grand mean | -0.249 | (37.78) | -0.554 | (25.27) |

#### 4.3.1.3 Combined analysis of the take-all susceptibility across the two field seasons

A combined analysis of the 2013-2014 take-all disease data and the 2014-2015 take-all disease data was performed using a REML analysis. Significant differences were found for the take-all disease data across the *T. monococcum* MDR037 (S) X MDR046 (R) mapping population (Table 4.6, $P$ = <0.001). The SED for the combined year analysis is smaller than the SED for the each of the individual years (Table 4.5 & Table 4.6), illustrating a more precise estimate of the treatment effects. The mapping population line 51 was ranked the most resistant line to take-all across the two years with line 44 ranked the second most resistant and the parental line MDR046 ranked as the third most resistant take-all line in the trials (Table 4.6). The mapping population line 54 was ranked the most susceptible line to take-all across the two years, with the parental line MDR037 having a ranking of 9$^{th}$ most susceptible to take-all. The highly susceptible control variety Hereward had an intermediate ranking of take-all susceptibility across all of the screened *T. monococcum* lines.

Table 4.6. Combined year analysis of the susceptibility of the *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population lines and controls to take-all disease for the 2013-2014 (2014/R/WW/1418) and 2014-2015 (2015/R/WW/1512) field trials.

| Treatment | Logit of take-all index (0-100), (back transformed means) | |
|---|---|---|
| **Controls** | | |
| MDR037 | -0.168 | (41.19) |
| MDR046 | -0.723 | (18.57) |
| Hereward | -0.378 | (31.47) |
| ***T. m* MDR037 X MDR046 mapping population lines** | | |
| Line 51 | -0.732 | (18.29) |
| Line 44 | -0.729 | (18.39) |
| Line 69 | -0.664 | (20.44) |
| Line 3 | -0.641 | (21.23) |
| Line 1 | -0.639 | (21.28) |
| Line 10 | -0.633 | (21.50) |
| Line 48 | -0.622 | (21.86) |
| Line 6 | -0.619 | (21.99) |
| Line 14 | -0.616 | (22.07) |
| Line 19 | -0.604 | (22.51) |
| Line 2 | -0.573 | (23.64) |
| Line 72 | -0.565 | (23.92) |
| Line 41 | -0.564 | (23.96) |
| Line 63 | -0.548 | (24.56) |
| Line 68 | -0.539 | (24.88) |
| Line 43 | -0.532 | (25.15) |
| Line 16 | -0.527 | (25.36) |
| Line 79 | -0.520 | (25.62) |
| Line 26 | -0.515 | (25.80) |
| Line 23 | -0.500 | (26.39) |
| Line 64 | -0.492 | (26.71) |
| Line 30 | -0.486 | (26.95) |
| Line 46 | -0.486 | (26.95) |
| Line 9 | -0.485 | (27.01) |
| Line 58 | -0.484 | (27.02) |
| Line 31 | -0.483 | (27.06) |
| Line 55 | -0.481 | (27.16) |
| Line 27 | -0.470 | (27.59) |
| Line 34 | -0.470 | (27.61) |
| Line 71 | -0.468 | (27.66) |
| Line 67 | -0.467 | (27.70) |
| Line 74 | -0.464 | (27.82) |

Table 4.6. Continued.

| Treatment | Logit of take-all index (0-100), (back transformed means) | |
|---|---|---|
| ***T. m* MDR037 X MDR046 mapping population lines** | | |
| Line 50 | -0.459 | (28.04) |
| Line 12 | -0.430 | (29.24) |
| Line 38 | -0.426 | (29.40) |
| Line 56 | -0.417 | (29.76) |
| Line 21 | -0.412 | (30.00) |
| Line 39 | -0.402 | (30.42) |
| Line 28 | -0.402 | (30.42) |
| Line 62 | -0.400 | (30.49) |
| Line 22 | -0.386 | (31.10) |
| Line 17 | -0.380 | (31.36) |
| Line 25 | -0.373 | (31.66) |
| Line 61 | -0.373 | (31.69) |
| Line 66 | -0.363 | (32.12) |
| Line 5 | -0.350 | (32.66) |
| Line 76 | -0.350 | (32.67) |
| Line 80 | -0.344 | (32.95) |
| Line 37 | -0.341 | (33.07) |
| Line 59 | -0.340 | (33.13) |
| Line 73 | -0.339 | (33.18) |
| Line 70 | -0.339 | (33.19) |
| Line 78 | -0.330 | (33.56) |
| Line 83 | -0.327 | (33.70) |
| Line 82 | -0.326 | (33.74) |
| Line 47 | -0.323 | (33.91) |
| Line 65 | -0.318 | (34.12) |
| Line 57 | -0.316 | (34.21) |
| Line 53 | -0.316 | (34.21) |
| Line 20 | -0.308 | (34.58) |
| Line 24 | -0.298 | (35.04) |
| Line 60 | -0.276 | (36.02) |
| Line 45 | -0.257 | (36.94) |
| Line 36 | -0.255 | (37.01) |
| Line 7 | -0.253 | (37.12) |
| Line 13 | -0.244 | (37.52) |
| Line 75 | -0.239 | (37.75) |
| Line 81 | -0.220 | (38.66) |
| Line 77 | -0.218 | (38.78) |
| Line 4 | -0.213 | (39.00) |
| Line 33 | -0.189 | (40.17) |

Table 4.6. Continued.

| Treatment | Logit of take-all index (0-100), (back transformed means) | |
|---|---|---|
| ***T. m* MDR037 X MDR046 mapping population lines** | | |
| Line 52 | -0.166 | (41.29) |
| Line 29 | -0.149 | (42.11) |
| Line 11 | -0.125 | (43.29) |
| Line 42 | -0.118 | (43.61) |
| Line 40 | -0.108 | (44.11) |
| Line 15 | -0.107 | (44.19) |
| Line 35 | -0.058 | (46.63) |
| Line 54 | 0.028 | (50.88) |
| d.f. | 81 | |
| SED | 0.181 | |
| F statistic | 1.69 | |
| F probabilty | <0.001 | |
| Grand mean | -0.397 | (31.05) |

#### 4.3.1.4 Above-ground phenotypic crop measurements of the 2013-2014 field trial experiment

The above-ground phenotypic crop measurements for the 2013-2014 field trial (2014/R/WW/1418) indicated that there were significant effects of genotype on days to anthesis (*P* = <0.001), days to leaf senescence (*P* = <0.001) and plant height (*P* = <0.001) (Table 4.7). Days to anthesis (from 01/05/14) ranged from 48.74 - 56.27 days for *T. monococcum* MDR037 (S) X MDR046 (R) mapping population lines and parental lines (Table 4.7). The hexaploid wheat variety Hereward reached anthesis earlier, 43.40 days from 01/05/14. Days to leaf senescence (from 01/06/14) ranged from 43.29 - 49.47 for the mapping population lines and parental lines (Table 4.7). The hexaploid control variety Hereward senesced later than the *T. monococcum* lines. Plant height ranged from 94.3 cm - 128.8 cm for the mapping population lines and parental lines. An expected shorter plant height of 80.7 cm was found for the modern hexaploid wheat control variety Hereward.

The above-ground phenotypic crop measurements were correlated against one another. A weak positive correlation was found between days to anthesis and days to leaf senescence ($r_s$ = 0.246, *P* = 0.034, n = 75). There was no significant correlation between plant height and days to leaf senescence ($r_s$ = -0.203, *P* = 0.080,

n = 75) and no significant correlation between plant height and days to anthesis ($r_s$ = -0.110, *P* = 0.348, n = 75). The above-ground phenotypic crop measurements were then correlated against the take-all indexes for each line. A weakly negative correlation was found between take-all index and days to leaf senescence ($r_s$ = -0.284, *P* = 0.014, n = 75; Figure 4.9). The earlier that the leaves senesced, the higher the take-all index (Figure 4.9). There was no significant correlation between take-all index and days to anthesis ($r_s$ = 0.051, *P*= 0.666, n = 75) and no significant correlation between take-all index and plant height ($r_s$ = 0.117, *P* = 0.318, n = 75). The plant height of the *T. monococcum* lines per plot, excluding Hereward, were also plotted against the establishment of the field trial (Figure 4.10). There was a trend for the *T. monococcum* plots that were in the flooded area of the field trial and therefore a very poor germination rate, to have a shorter plant height (Figure 4.10).

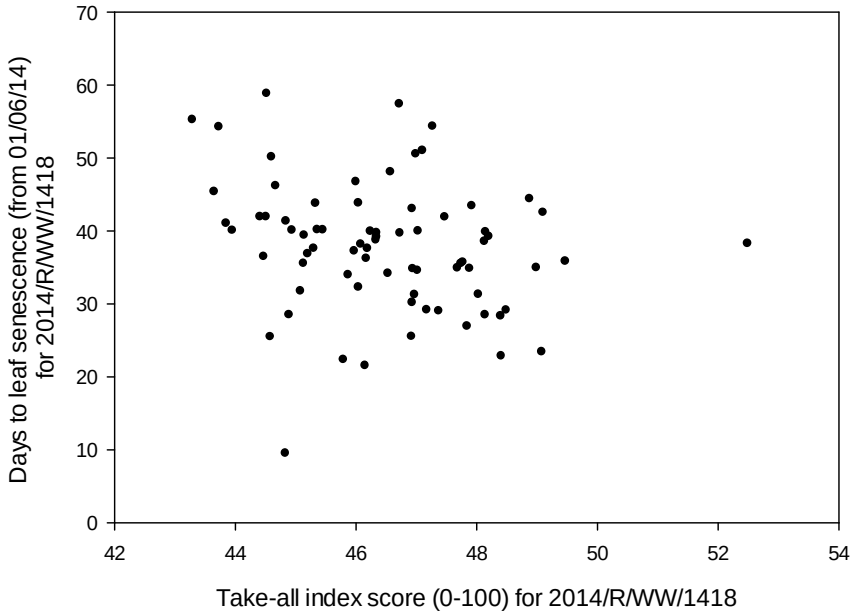



Figure 4.9. Correlation between take-all index score (0-100) and days to leaf senescence (from 01/06/14) for 2013-2014 (2014/R/WW/1418) field trial. $r_s$ = -0.284, *P* = 0.014, n = 75.

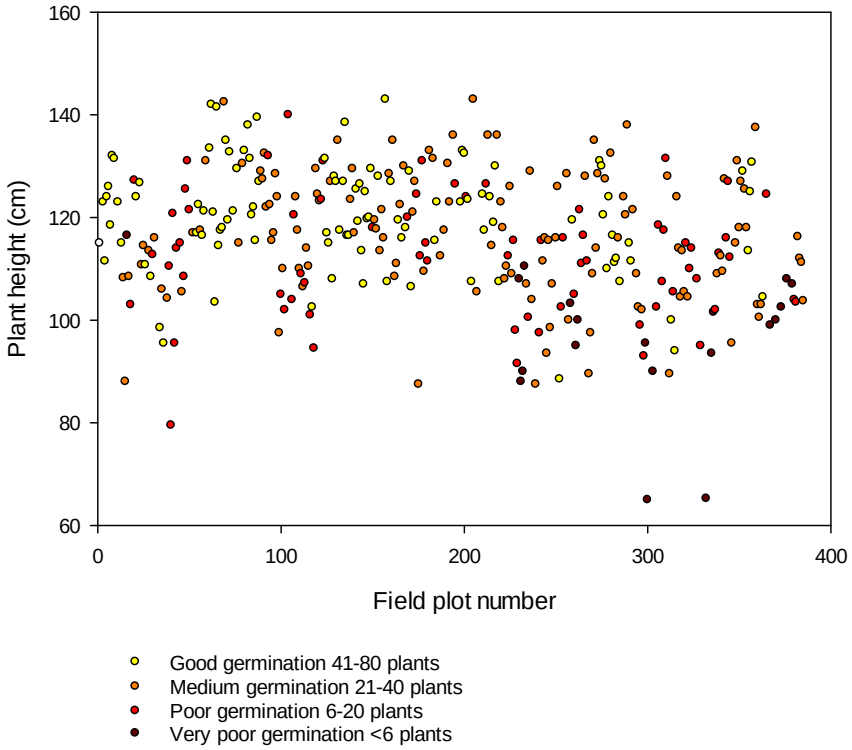



Figure 4.10. Plant height (cm) of the *Triticum monococcum* lines for the 2013-2014 (2014/R/WW/1418) field trial plotted against the establishment of their field trial plot.

#### 4.3.1.5 Above-ground phenotypic crop measurements of the 2014-2015 field trial experiment

The above-ground phenotypic crop measurements for the 2014-2015 field trial (2015/R/WW/1513) also indicated that there were significant effect of genotype on days to anthesis ($P$ = <0.001), days to leaf senescence ($P$ = <0.001) and plant height ($P$ = <0.001) (Figure 4.10). Days to anthesis (from 01/05/15) had a larger range for the *T. monococcum* mapping population lines and parental lines of just under 10 days compared to a 7 day range in 2013-2014. The hexaploid wheat variety Hereward again reached anthesis earlier than the *T. monococcum* lines and at an earlier date than in the 2013-2014 field season (2015: 9$^{th}$ June; 2014: 13$^{th}$ June). Days to leaf senescence (from 01/06/15) also had a slightly larger range for the *T. monococcum* mapping population lines and parental lines of 8 days compared to 6 day range in 2013-2014. The leaves also began to reach senescence five days earlier

for the hexaploid wheat variety Hereward then seen in 2013-2014. Plant height had a smaller range of 105.8 - 128.3 cm for the mapping population lines and parental lines and the grand mean of the height of plants across the field trial was 5 cm higher than the grand mean in 2014. Again an expected shorter plant height of 81 cm was found for the modern hexaploid wheat control variety Hereward.

When the above-ground phenotypic crop measurements were correlated against one another, a moderately positive correlation was found between the days to anthesis and the days to leaf senescence ($r_s$ = 0.479, *P* = <0.001, n = 82). A moderately negative correlation was found between the days to leaf senescence and plant height ($r_s$ = -0.399, *P* = <0.001, n = 82). No significant correlation was found between days to anthesis and plant height ($r_s$ = 0.127, *P* = 0.256, n = 82). The above-ground phenotypic crop measurements were then correlated against the take-all indexes for each line. Unlike the 2013-2014 field trial data, there was no significant correlation found between days to leaf senescence and TAI ($r_s$ = -0.185, *P* = 0.096, n = 82), yet a weak negative correlation was found between the days to anthesis and TAI ($r_s$ = -0.284, *P* = 0.010, n = 82). No significant correlation was found between plant height and TAI ($r_s$ = -0.052, *P* = 0.643, n = 82).

The above-ground phenotypic crop measurements from the 2013-2014 field trial were then correlated with the above-ground phenotypic crop measurements from the 2014-2015 field trial. A moderately positive correlation was found between the days to anthesis in 2013-2014 and the days to anthesis in 2014-2015 ($r_s$ = 0.341, *P* = 0.003, n = 75). A weak positive correlation was found between the days to senescence in 2014 and the days to senescence in 2015 ($r_s$ = 0.277, *P* = 0.016, n = 75). A strong positive correlation was found between the recorded plant height in 2013-2014 and the recorded plant height in 2014-2015 ($r_s$ = 0.700, *P* = <0.001, n = 75). During 2013-2014 the height of the resistant parental *T. monococcum* MDR046 accession was greater than the susceptible parental *T. monococcum* MDR037 accessions, although this reversed during the 2014-2015 field season (Figure 4.10).

Table 4.7. Above-ground phenotypic crop measurements of days to anthesis, days to leaf senescence and plant height of *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population lines and controls for the 2013-2014 (2014/R/WW/1418) and 2014-2015 (2015/R/WW/1512) field trials.

| Treatment | Days to anthesis | | Days to leaf senescence | | Plant height (cm) | |
|---|---|---|---|---|---|---|
| | Date from 1st May | | Date from 1st June | | | |
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| **Controls** | | | | | | |
| MDR046 | 51.51 | 49.17 | 46.92 | 45.66 | 124.7 | 119.9 |
| MDR037 | 53.89 | 48.92 | 49.10 | 46.06 | 119.0 | 121.8 |
| Hereward | 43.40 | 39.08 | 52.49 | 47.58 | 80.7 | 81.0 |
| ***Triticum monococcum* MDR037 X MDR046 mapping population lines** | | | | | | |
| Line 1 | 50.68 | 50.05 | 46.15 | 45.09 | 116.9 | 123.9 |
| Line 2 | 51.93 | 49.81 | 46.08 | 48.55 | 105.8 | 111.5 |
| Line 3 | 51.12 | 48.44 | 48.20 | 45.89 | 119.2 | 120.7 |
| Line 4 | 53.68 | 51.05 | 46.99 | 44.31 | 120.7 | 123.9 |
| Line 5 | 54.93 | 52.80 | 45.33 | 44.62 | 121.2 | 122.7 |
| Line 6 | 53.46 | 49.28 | 47.17 | 49.17 | 113.0 | 113.1 |
| Line 7 | 49.87 | 46.61 | 45.97 | 46.04 | 106.3 | 111.8 |
| Line 9 | 52.83 | 46.70 | 45.45 | 43.47 | 104.9 | 116.8 |
| Line 10 | 53.61 | 48.86 | 47.02 | 45.24 | 111.1 | 125.5 |
| Line 11 | | 48.35 | | 43.42 | | 111.4 |
| Line 12 | 52.38 | 48.89 | 48.15 | 45.86 | 110.2 | 116.0 |
| Line 13 | | 46.88 | | 44.06 | | 109.8 |
| Line 14 | 53.76 | 54.35 | 46.93 | 45.64 | 109.7 | 119.2 |
| Line 15 | 53.68 | 55.17 | 47.27 | 47.51 | 116.1 | 122.6 |
| Line 16 | 50.61 | 51.89 | 48.49 | 48.43 | 104.9 | 114.5 |
| Line 17 | 54.19 | 46.48 | 47.92 | 42.93 | 113.2 | 122.4 |
| Line 19 | 54.92 | 50.36 | 48.40 | 49.72 | 104.8 | 112.4 |
| Line 20 | 54.27 | 48.96 | 49.08 | 47.43 | 114.6 | 114.5 |
| Line 21 | 52.05 | 47.89 | 47.68 | 44.36 | 124.6 | 127.8 |
| Line 22 | 51.12 | 48.93 | 45.13 | 46.14 | 107.9 | 121.0 |
| Line 23 | 53.90 | 54.49 | 45.20 | 48.62 | 115.0 | 115.3 |
| Line 24 | 52.32 | 48.93 | 46.04 | 46.84 | 102.3 | 114.5 |
| Line 25 | 53.70 | 49.77 | 46.34 | 48.55 | 105.9 | 113.8 |
| Line 26 | 56.27 | 52.91 | 46.17 | 47.16 | 113.5 | 125.1 |
| Line 27 | 53.29 | 53.76 | 44.58 | 45.83 | 119.9 | 124.3 |
| Line 28 | 51.19 | 48.35 | 46.33 | 46.74 | 104.9 | 114.2 |
| Line 29 | 52.10 | 52.85 | 43.29 | 46.77 | 113.1 | 119.3 |
| Line 30 | 51.78 | 48.35 | 48.99 | 48.87 | 94.3 | 106.9 |

Table 4.7. Continued.

| Treatment | Days to anthesis | | Days to leaf senescence | | Plant height (cm) | |
|---|---|---|---|---|---|---|
| | Date from 1st May | | Date from 1st June | | | |
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| ***Triticum monococcum* MDR037 X MDR046 mapping population lines** | | | | | | |
| Line 31 | 51.04 | 50.62 | 44.89 | 45.67 | 122.1 | 125.6 |
| Line 33 | 50.83 | 46.18 | 44.67 | 42.15 | 123.5 | 128.3 |
| Line 34 | 51.14 | 48.88 | 46.53 | 47.59 | 110.2 | 117.1 |
| Line 35 | 52.75 | 46.55 | 44.52 | 42.93 | 127.7 | 117.9 |
| Line 36 | 54.37 | 48.87 | 46.24 | 44.77 | 109.2 | 124.3 |
| Line 37 | 54.67 | 52.16 | 48.88 | 47.00 | 112.8 | 115.1 |
| Line 38 | 50.80 | 49.22 | 48.41 | 43.30 | 116.7 | 122.8 |
| Line 39 | 52.21 | 48.33 | 47.77 | 43.57 | 124.4 | 127.8 |
| Line 40 | 50.47 | 46.49 | 46.72 | 43.32 | 120.9 | 119.3 |
| Line 41 | 51.46 | 48.71 | 43.95 | 44.50 | 115.2 | 124.1 |
| Line 42 | | 51.61 | | 49.31 | | 109.8 |
| Line 43 | 51.47 | 48.76 | 47.37 | 44.41 | 116.5 | 118.4 |
| Line 44 | 51.29 | 49.53 | 44.83 | 44.49 | 114.7 | 116.2 |
| Line 45 | 50.14 | 49.18 | 44.60 | 46.27 | 111.9 | 120.5 |
| Line 46 | 50.79 | 48.42 | 48.14 | 47.97 | 106.7 | 109.3 |
| Line 47 | 51.72 | 46.71 | 46.73 | 42.78 | 116.6 | 128.1 |
| Line 48 | 54.22 | 54.42 | 48.13 | 48.11 | 118.0 | 123.2 |
| Line 50 | 52.18 | 49.86 | 47.74 | 45.61 | 116.7 | 120.0 |
| Line 51 | 50.95 | 50.62 | 45.79 | 45.24 | 116.9 | 120.0 |
| Line 52 | | 51.47 | | 46.77 | | 118.4 |
| Line 53 | 51.18 | 49.86 | 45.30 | 48.41 | 115.8 | 114.3 |
| Line 54 | 52.67 | 44.65 | 46.57 | 45.32 | 102.8 | 105.8 |
| Line 55 | 50.90 | 53.32 | 47.88 | 48.29 | 127.7 | 123.7 |
| Line 56 | 52.61 | 48.32 | 45.08 | 45.08 | 110.1 | 122.4 |
| Line 57 | 50.28 | 48.37 | 45.14 | 43.80 | 118.4 | 122.4 |
| Line 58 | 54.68 | 48.97 | 45.87 | 47.01 | 113.5 | 116.9 |
| Line 59 | 52.96 | 45.40 | 44.94 | 44.88 | 113.8 | 115.7 |
| Line 60 | 48.74 | 46.15 | 43.73 | 43.61 | 119.3 | 116.8 |
| Line 61 | 51.88 | 52.04 | 47.47 | 50.22 | 108.9 | 122.3 |
| Line 62 | 54.22 | 48.87 | 49.47 | 45.42 | 99.6 | 115.0 |
| Line 63 | 51.24 | 49.96 | 46.94 | 48.43 | 108.9 | 119.3 |
| Line 64 | 51.70 | 54.19 | 44.47 | 45.77 | 123.2 | 117.8 |
| Line 65 | 52.57 | 51.43 | 46.04 | 46.30 | 101.2 | 112.9 |
| Line 66 | 51.27 | 46.32 | 44.84 | 45.55 | 113.7 | 117.7 |
| Line 67 | 52.26 | 48.97 | 43.85 | 42.75 | 111.5 | 116.8 |
| Line 68 | 53.23 | 49.09 | 47.03 | 44.82 | 112.2 | 121.3 |
| Line 69 | 51.48 | 49.09 | 47.84 | 43.81 | 109.7 | 115.2 |

Table 4.7. Continued.

| Treatment | Days to anthesis | | Days to leaf senescence | | Plant height (cm) | |
|---|---|---|---|---|---|---|
| | Date from 1st May | | Date from 1st June | | | |
| | 2014 | 2015 | 2014 | 2015 | 2014 | 2015 |
| ***Triticum monococcum* MDR037 X MDR046 mapping population lines** | | | | | | |
| Line 70 | 50.78 | 48.98 | 44.51 | 43.66 | 130.9 | 125.3 |
| Line 71 | 54.06 | 52.87 | 46.34 | 44.66 | 119.6 | 128.2 |
| Line 72 | 49.54 | 49.51 | 46.19 | 44.31 | 119.8 | 125.5 |
| Line 73 | 52.10 | 51.01 | 47.10 | 47.41 | 120.8 | 126.9 |
| Line 74 | 52.74 | 47.21 | 48.03 | 44.02 | 114.8 | 121.0 |
| Line 75 | 50.99 | 46.34 | 46.00 | 43.68 | 115.6 | 121.0 |
| Line 76 | 50.65 | 47.84 | 43.65 | 46.10 | 102.9 | 109.5 |
| Line 77 | | 45.75 | | 42.83 | | 124.0 |
| Line 78 | | 48.49 | | 47.50 | | 123.5 |
| Line 79 | 50.38 | 48.73 | 46.97 | 45.65 | 109.6 | 113.3 |
| Line 80 | 51.81 | 47.91 | 45.36 | 44.75 | 114.4 | 118.1 |
| Line 81 | 54.24 | 50.69 | 46.93 | 45.16 | 115.7 | 119.9 |
| Line 82 | 50.26 | 46.64 | 44.41 | 44.93 | 128.8 | 125.9 |
| Line 83 | | 48.02 | | 48.41 | | 117.2 |
| d.f. | 74 | 81 | 74 | 81 | 74 | 81 |
| SED | 1.379 | 0.876 | 1.551 | 1.197 | 5.172 | 3.044 |
| F statistic | 11.69 | 39.73 | 5.97 | 5.57 | 14.36 | 36.1 |
| F probabilty | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Grand mean | 52.08 | 49.27 | 46.49 | 45.79 | 113.63 | 118.53 |

## 4.4 Discussion

### 4.4.1 Response of *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population to take-all disease in the field

Two field trials were carried out to evaluate the resistance of the *T. monococcum* MDR037 (S) X MDR046 (R) mapping population to take-all disease across two field seasons. The field trial carried out in the 2013-2014 season was under a moderate disease pressure, whereas the 2014-2015 season exhibited a slightly lower disease pressure year. Both of the field trials revealed differences in take-all disease across the mapping population lines, with a good spread of susceptibility to take-all, although these differences were not found to be statistically significant during the 2013-2014 field trial. Spatially adjusted models were used on the analysis of the field trial data, suggesting that, as expected, take-all was not uniform throughout the field trial sites.

Differences were found for the two *T. monococcum* parental lines in their susceptibility to take-all infection across the two field seasons. During the higher disease pressure year of 2013-2014, there was less difference in the TAI scores between the moderately resistant parental line MDR046 and the susceptible parental line MDR037. This suggests that the partial resistance is more prominent when the take-all disease severity is lower. A similar trend was found in Chapter 3, the moderately resistant hexaploid varieties indicated a greater level of take-all resistance during the lower disease pressure field season of 2013-2014. The two field trials, within this chapter, were however carried out in different fields across the Rothamsted Farm and the different soil type and conditions along with the different take-all population structures populations, as discussed in Chapter 1 (Bateman et al., 1997), may have affected the take-all disease severity between the two years. The combined year analysis revealed that the *T. monococcum* MDR037 (S) X MDR046 (R) mapping population line 51 ranked the most resistant to natural populations of take-all, closely followed by line 44 and the moderately resistant parental line MDR046. However, line 51 indicated more of a consistent level of resistance to take-all disease across the two different field seasons under differing environmental conditions.

Above-ground phenotypic crop measurements were recorded across both field seasons. Significant correlations for each of the above-ground measurements were found between the 2013-2014 data and 2014-2015 data, suggesting that there are some consistencies in these above ground measurements across the *T. monococcum* mapping population for both field seasons. Lines that reached anthesis first and lines that reached leaf senescence first were significantly, yet weakly to moderately, correlated across the two years. Differences in the date that lines reached anthesis and leaf senescence were expected due to varying environmental conditions across the two field seasons and different sowing dates; the 2014-2015 field trial was sown a month earlier than the 2014-2015 field trial. When analysing the above-ground phenotypic crop measurements, it was apparent that the hexaploid wheat cultivar Hereward showed differing trends, as expected, compared to the diploid *T. monococcum* mapping population. Leaf senescence was seen to be negatively correlated with TAI score in the 2013-2014 field trial, suggesting that either plots with a high take-all disease pressure became stressed and senesced earlier than plots with a lower disease pressure or that the earlier senescing lines were more susceptible to take-all disease. The strength of the relationship of the correlation was only moderate. However, to test these differing hypotheses leaf senescence would need to be scored for the *T. monococcum* mapping population in a take-all free 1$^{st}$ wheat situation. Days to anthesis was seen to be negatively correlated to TAI score in the 2014-2015 field trial, suggesting that plots with a high take-all disease pressure reached anthesis earlier than plots with a lower take-all disease pressure. The strength of the relationship of the correlation was weak. These findings suggest that it would be difficult to use above-ground measurements as a visual field scoring system for take-all disease in the field, as previously found in Chapter 3. A take-all patch score has previously been devised to visually assess and score the percentage of prematurely ripened and stunted plants, caused by take-all infection, within an area (Gutteridge et al., 2006). A number of problems can arise with this method. Take-all severity may be low, with a lack of prematurely ripened and stunted plants, as well as a hot/dry weather period causing premature ripening of the crop, affecting the visual screening of take-all patches. The same problems could easily arise when visually assessing leaf

senescence as an indicator of take-all disease. These results highlight that taking plant samples and assessing roots for take-all infection is currently the most efficient method to screen large amount of germplasm in the field. In these small plot trials due lack of available seed, take-all patch score analysis was not possible.

The environmental conditions varied across the two years, which would have influenced the severity of take-all disease in the field between the two field seasons as well as affecting the above-ground phenotypic crop measurements. The 2013-2014 field season started off with a mild and wet winter. The total rainfall across December and January was over double the amount present in the 2014-2015 field season. The 2013-2014 field trial site became flooded and was under a considerable amount of water from January through to the beginning of March, resulting in poor establishment of a number of plots. A shorter plant height grand mean was found for the 2013-2014 field trial and there was a trend for the very poorly germinated plots to have a shorter plant height. A total of 24 plots had a very poor establishment with less than 6 plants per plot, with block three and four the worse affected. It was thought that the plots with very poor establishment would not accurately represent the take-all disease pressure in the soil under those plots as the structure of the adult plant root systems would differ from the lack of neighbouring wheat plants within the plot. During the statistical analysis, these plots were inputted as missing values and it was decided that there was no need to combine block three and block four of the design replication due to a high number of replication of the mapping population lines throughout the trial.

It is also not known how the take-all fungus would react to being submerged in water for such a long period of time. A number of studies have looked at the saprophytic survival of the take-all fungus under dry and wet soil conditions (Wong, 1984). It is however unclear how the take-all fungus would survive during extremely wet soil conditions once the crop had been sown and once primary infection of the plant had occurred. However, there appeared to be no trend in the establishment of the plots and their corresponding TAI score. Within the flooded field trial, the take-all fungus would have colonised the wheat roots and therefore the fungus may have been partially protected from the waterlogged soil. This suggest that the

flooding event had more of an effect on the development of the plant and that this may have had the potential for subsequent knock-on effects on take-all disease levels. Interestingly, the long-term storage of take-all isolates consist of placing a 1 $cm^2$ agar plug in sterile distilled water.

A higher level of rainfall was found across the months of March through to June in 2014 compared to rainfall levels in 2015. Temperature can also affect the severity of take-all disease. Dry weather during the summer months can exacerbate the above-ground effects of take-all disease on a crop since these dry periods can be sufficient in putting the crop under moisture stress, leading to the development of take-all patches when the disease levels are only moderate (Hornby et al., 1998). The 2013-2014 field season saw a warmer and drier June to July compared to slightly milder and extremely wet July during the 2014-2015 field season. The rainfall later in the season would have influenced the differences found between the take-all severity across the two field seasons since a wet spring can encourage take-all disease development (Hornby et al., 1998). Throughout the spring and summer months, winter wheat undergoes a number of developmental stages over a short period of time and any short-term weather events can be crucial in the development of the plant (Hornby et al., 1998).

A pot bioassay was set up along with Gail Canning in order to compare the susceptibility of *T. monococcum* accessions to take-all disease at the seedling stage with their susceptibility to take-all under field conditions (see Appendix 4.4 - 4.6). Gail Canning assessed the pot bioassay seedling roots and found that the susceptibility of the parental lines of the MDR037 (S) X MDR046 (R) mapping population to take-all infection was not comparable to the field performance of the lines. *Triticum monococcum* accession MDR043 indicated the lowest percentage of take-all infected roots in the pot bioassay and MDR037 the highest, yet field trials have shown that both of these accessions are fully susceptible to take-all disease (Appendix 4.6) (Chapter 3) (McMillan et al., 2014). However, *T. monococcum* accessions MDR031 and MDR046 are moderately resistant to take-all disease in the field (Chapter 3) (McMillan et al., 2014), with both accessions having a higher percentage of take-all infected roots than accession MDR043. This confirms that it

would not have been possible to screen the *T. monococcum* MDR037 (S) X MDR046 (R) mapping population via a pot bioassay.

It is important to assess the diversity of wild relatives, in particular *T. monococcum*, at the genome level in order to utilise the germplasm in modern wheat genetic improvement programmes (Jing et al., 2009). The genetic diversity of *T. monococcum* has previously been studied by Diversity Array Technology (DArT) (Jing et al., 2009, McMillan et al., 2014). DArT is a high throughput genome analysis that scores for the presence or absence of DNA fragments from the total genomic DNA of an organism or a population of organisms (Jaccoud et al., 2001). Jing et al. (2009) initially developed DArT markers for *T. monococcum*. McMillan et al. (2014) have since carried out DArT genotyping to include *T. monococcum* accessions that had been found to exhibit moderate to high levels of resistance to take-all disease. Moderately resistant and susceptible *T. monococcum* accessions were found to cluster together and the study revealed that whole genome diversity was not associated to the susceptibility of the accessions to take-all. The most take-all resistant *T. monococcum* accessions, MDR031 and MDR046, did however form a cluster together. The two accessions were collected by the Vavilov Institute in Russia and were collected from differing geographical locations, MDR031 has an origin of Turkey and MDR046 an origin of Romania. McMillan et al. (2014) therefore suggested that multiple genetic sources of take-all resistance may occur within *T. monococcum*

The mechanism of the partial resistance to take-all disease in *T. monococcum* is unknown and could be either tissue-based resistance or disease escape. Rye is highly resistant to take-all disease (Nilsson, 1969, Scott, 1981, Rothrock, 1988, Bithell et al., 2011) and has been found to exhibit disease escape, by producing new roots, as well as displaying tissue-based resistance (Skou, 1975). Within this PhD study, root counts were performed in the pot bioassay that screened the 34 *T. monococcum* accessions for susceptibility to take-all and was assessed by Gail Cannning (see Appendix 4.4-4.6). The take-all susceptible *T. monococcum* mapping population parental line MDR037 had the highest number of roots per plant, suggesting that at the seedling stage the take-all partially resistant mapping

population parental line MDR046 does not produce more roots as a form of disease escape (Appendix 4.6). Root counts were not performed on the field trials within this chapter, but no noticeable differences in the structure of the adult plant root systems were seen when visually assessing the roots by eye for take-all infection. The *T. monococcum* parental lines of the MDR037 (S) X MDR046 (R) mapping population, along with *T. monococcum* parental lines of the MDR043 (S) X MDR031 (R) mapping population (not screened within this PhD project), were however included in the 2014 field trial that screened hexaploid wheat varieties possessing a level of resistance to take-all disease (2014/R/WW/1419) in Chapter 3. This field trial was also in the same field as the 2014 field trial screening the *T. monococcum* MDR037 (S) X MDR046 (R) mapping population. Seminal root counts, crown root counts and tiller counts were performed in this trial and analysis of these measurements revealed that there was no trend for the number of seminal roots across the take-all resistant or susceptible *T. monococcum* accessions (number of seminal roots per plant: MDR046 = 4.49, MDR031 = 4.49, MDR037 = 4.84, MDR043 = 3.84) (Chapter 3). The *T. monococcum* accession MDR037 had a higher number of crown roots than the three other accessions (number of crown roots per plant: MDR046 = 30.47, MDR031 = 33.18, MDR037 = 38.07, MDR043 = 33.18) (Chapter 3). There appeared to be no trend for the number of tillers across the take-all resistant or susceptible *T. monococcum* accessions (number of tillers per plant: MDR046 = 10.69, MDR031 = 12.89, MDR037 = 10.82, MDR043 = 11.76) (Chapter 3). The *T. monococcum* parental line MDR037 had the highest number of crown roots, suggesting that the results from the seedling plants from the pot bioassay in this chapter and the adult plants from the field trial (2014/R/WW/1419) in Chapter 3 correspond. In the future root counts could be carried out on the adult plant root systems of the MDR037 (S) X MDR046 (R) mapping population to determine whether any lines that have partial resistance to take-all also produce a higher number of crown roots than those lines that are susceptible to take-all. To fully test this the lines would also need to be sown in a 1$^{st}$ wheat field trial to assess root production in the absence of take-all root infection in comparison to the 3$^{rd}$ wheat trial. However, these initial results suggest that *T. monococcum* is not exhibiting

disease escape as the partially resistant accessions are not producing a greater number of roots than the susceptible accessions.

Plant extracts have been suggested as an inhibitor of take-all disease. Wilkes et al. (1999) suggested that it could be hydroxamic acid found in cereal roots, such as rye, that inhibits take-all growth. The authors found that rye and triticale had both 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA), whereas only DIMBOA was present in wheat roots. DIBOA was found to be the most effective inhibitor of take-all and demonstrated a fungistatic effect on growth. The partial resistance found in in *T. monococcum* to take-all could be due to compounds inhibiting the growth of the fungus in the roots.

### 4.4.2 Further work

*Triticum monococcum* exhibits partial resistance to take-all and the initial hypothesis at the beginning of this PhD project was that the trait would be controlled by a number of genes or regions, unlike the *Triticum monococcum*-*Zymoseptoria tritici* interaction (Jing et al., 2008). The *T. monococcum* MDR037 (S) X MDR046 (R) mapping population was sent to the Bristol Genomics Facility at the University of Bristol for genotyping via microarray analysis using the Axiom® Wheat Breeder's Genotyping Array. The genotyping data was returned at the end of August 2016. Due to time constraints, data analysis was not included in the thesis. Results from a QTL analysis could provide novel information that will allow wheat breeders to explore whether it is possible to introgress the *T. monococcum* partial resistance to take-all into hexaploid wheat. The analysis could identify genetic markers and if tightly linked, could be potentially be used for marker assisted breeding programmes to screen plants for take-all resistance and to produce new varieties. Marker assisted selection (MAS) has a number of advantages over traditional phenotypic selection, the main advantages being that it saves time and is less labour intensive than screening for phenotypes such as disease resistance in the field. Partial resistance to *T. monococcum* does not appear in pot bioassay and can only be found in adult plants screened in the field. A disadvantage with marker-assisted selection is that only a proportion of the genetic variation is captured by the markers.

The field screening of the *T. monococcum* MDR037 (S) X MDR046 (R) mapping population is currently being replicated for a third year. Although this field trial is outside of my PhD project, the trial will be used to validate and confirm any identified QTLs for a third field season. A multi-environment QTL analysis will allow us to combine data from the three field trials. Environmental conditions can affect the levels of expression of quantitative traits (Collard *et al.* 2005). Our study is replicated over different field sites and different years which may influence the QTLs affecting our trait for take-all resistance. It is crucial to consider the QTL X environment interaction in our analysis and to determine whether the QTLs are stable. George *et al.* (2003) found significant QTL X environment interactions for QTLs conferring resistance to downy mildews in maize, proposing the expressions of the QTLs could be environment-dependent. It would also be important, for future validation, to test the performance of the *T. monococcum* mapping population lines outside of the Rothamsted Farm as well as potentially outside of the UK in order to screen the lines under different soil types, soil conditions and environmental conditions.

### 4.4.3 Summary

In summary, this study has provided the first field screening of the *T. monococcum* MDR037 (S) X MDR046 (R) mapping population under natural populations of take-all. A range in susceptibility was found across the mapping population. More work is needed to understand the mechanism of the partial resistance to take-all disease found in *T. monococcum,* yet it is thought that the resistance is tissue-based. The *T. monococcum* mapping population has been genotyped by a microarray analysis using the Axiom® Wheat Breeder's Genotyping Array. An initial quantitative trait locus (QTL) analysis will be carried out over the next month to determine the genomic location of the genes responsible for the resistance to take-all. Genetic resistance provides a cheap, effective and environmentally friendly approach to control plant pathogens. The *T. monococcum* MDR037 (S) X MDR046 (R) mapping population has been screened during the 2016 field season, and although outside of the timeframe of my PhD, the field trial will be used to validate and confirm the identification of QTLs found from the two field trials.

# Chapter 5. Assembly and characterisation of a *Phialophora* isolate collection recovered from the Rothamsted Farm and generation of the draft genomes of three *Phialophora* species

## 5.1 Introduction

The identification of species within the *Gaeumannomyces-Phialophora* complex can be highly challenging and time consuming. At present, there are no methods for isolating *Phialophora* spp. from soil and current techniques involve isolating the fungi from infected roots of field-grown plants or growing susceptible plants as baits within infected soil, as seen with the isolation of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) (Hornby et al., 1998). Traditional diagnostic methods consist of visual assessment of morphological disease characteristics on wheat (Hornby et al., 1998). Pathogenicity tests, also known as seedling infection tests, are used to determine the identification of isolates (Hornby et al., 1998).

*Phialophora* species within the *Gaeumannomyces-Phialophora* complex consist of *Phialophora graminicola* (Walker, 1980) (teleomorph *Gaeumannomyces cylindrosporus* (Hornby et al., 1977)) and *Phialophora* sp. lobed hyphopodia (Deacon, 1974) (teleomorph *Gaeumannomyces graminis* var. *graminis* (Walker, 1972)) that were isolated from various grass species (Scott, 1970). Whereas *Phialophora radicicola* (Cain, 1952) was first isolated from maize (*Zea mays*) (McKeen, 1952). Previous work by Ward and Bateman (1999) found that ITS sequence analysis indicated that *P. radicicola* was identical to two pathogens that infect maize, *Gaeumannomyces graminis* var. *maydis* (*Ggm*) (Yao et al., 1992) and *Phialophora zeicola* (Deacon and Scott, 1983). There are two *Phialophora* species that occur naturally in the UK, *P. graminicola* and *Phialophora* sp. lobed hyphopodia (Deacon, 1973b), *Phialophora* sp. lobed hyphopodia is thought to be less common in the UK (Hornby et al., 1998). In addition, an unnamed *Phialophora* sp. has previously been isolated from the Rothamsted Farm (Ward and Bateman, 1999) (the unnamed *Phialophora* sp. has also been previously isolated in Germany (Ulrich et al., 2000)). *Phialophora graminicola* tends to cause roots to appear grey in colouration due to the build-up of mycelium and runner hyphae, whilst roots

appear a light to medium brown colouration with brown runner hyphae when infected with *Phialophora* sp. lobed hyphopodia (Hornby et al., 1998). *Phialophora* spp. develop subepidermal vesicles within roots with small, clustered vesicles illustrating *P. graminicola* and large single vesicles in side root epidermal cells for *Phialophora* sp. lobed hyphopodia (Hornby et al., 1998). These vesicles represent growth cessation immediately post penetration to prevent further growth (Deacon, 1974). *Gaeumannomyces graminis* var. *tritici* forms small unlobed hyphopodia and *Phialophora* sp. lobed hyphopodia forms large lobed hyphopodia on lower stem tissue, whereas *P. graminicola* does not grow at all on stem bases (Deacon, 1974).

*Phialophora* spp. can be isolated from roots using the same method described for *Ggt* (Hornby et al., 1998). *Phialophora* sp. lobed hyphopodia growth is visible on PDA plates after 2-4 days with similar coloration to *Ggt* but with more conspicuous aerial hyphae (Hornby et al., 1998). Growth of *P. graminicola* is visible after 5 days, a further 4 days reveals pigmentation ranging from orange to grey (Hornby et al., 1998). The problem of not being able to isolate *Phialophora* spp. from the soil may have led to the majority of studies being performed on a limited selection of culturable isolates recovered from baited plants.

A diverse array of molecular diagnostic methods is also available to differentiate species within the *Gaeumannomyces-Phialophora* complex but few elicit reliable specificity. Previous studies have used restriction fragment length polymorphisms to distinguish species but more recently a number of PCR primers have been developed. Bryan et al. (1995) developed primers that were used to distinguish between *Ggt* and *Gaeumannomyces graminis* var. *avenae* (*Gga*) but stated that they were unsure of primer specificity when tested on plants from the field in the presence of soil containing other fungi. Goodwin et al. (1995) designed specific primers based on the poorly conserved sequences of the internal transcribed spacers (ITS) 1 and 2 of *Gga* and *Gaeumannomyces graminis* var. *graminis* (*Ggg*) and stated that specificity was confirmed and *P. graminicola* and *Ggt* isolates were not amplified. Ward (1995) modified and specified a PCR assay using the primers KS1F and KS2R, previously published by Schesser et al. (1991), to detect *Gaeumannomyces graminis* species. However, Ward and Bateman (1999) found

that the primers provided a band of around 500 bp for the species *Phialophora radicicola*, *P. zeicola* and *Ggm*, similar to the 550 bp product size for *Ggg*. Rachdawong et al. (2002) differentiated *Gga*, *Ggg* and *Ggt* from one another using PCR amplification of avenacinase-like genes. All three of the variety-specific primers were used in one tube through the use of three variety-specific 5' primers and one common 3' primer. *Phialophora radicicola*, *Phialophora* sp. and *G. cylindrosporus* were not amplified with any of the variety-specific primers but a few of the *Ggt* isolates gave a positive result for *Ggg*. However, a study by Kwak et al. (2010) stated that they had been able to generate PCR products with the primers generated by Rachdawong et al. (2002) for a number of *Phialophora* isolates. Difficulties with specificity of primers has led to unidentified isolates of *Phialophora* in a number of studies as well as species with the *Gaeumannomyces-Phialophora* complex not being identified to the species level, leading to confusion within the literature (Freeman and Ward, 2004). Future work therefore needs to focus upon improving the specificity of primers in order to distinguish between species within the *Gaeumannomyces-Phialophora* complex. An in depth re-analysis of the species complex has recently been carried out, and is discussed in Chapter 7, in the laboratory of Dr Pedro Crous (CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands) with historic isolates having been supplied by Rothamsted Research (RRes) (Hernández-Restrepo et al., 2016).

Within the *Magnaporthe* comparative project, a collaboration between the International Rice Blast Genome Consortium and the Broad Institute, (http://www.broadinstitute.org/scientific-community/science/projects/fungal-genome-initiative/magnaporthe-comparative-genomics-proj) the genome of *Magnaporthe oryzae* (strain 70-15) (Dean et al., 2005, Okagaki et al., 2015) and the draft assemblies of the *Magnaporthe poae* (strain ATCC 64411) genome (Okagaki et al., 2015) and the *Gaeumannomyces graminis* var. *tritici* (*Ggt*) (isolate R3-111a-1) genome (Okagaki et al., 2015) have been sequenced and annotated at the Broad Institute (Table 5.1). The *M. orzyae* sequenced strain was a laboratory strain that was initially generated by crossing a rice isolate with a weeping love grass (*Eragrostis curvula*) isolate and backcrossing with the rice isolate Guy11 originating

from French Guyana (Chao and Ellingboe, 1991) and the genome was sequenced using Sanger sequencing, finished to seven chromosomes (Dean et al., 2005, Okagaki et al., 2015). The *M. poae* and *Ggt* genomes were sequenced using a combination of Sanger sequencing, 454 sequencing and Illumina sequencing, with *M. poae* sequenced to 40X coverage and *Ggt* sequenced to 25X coverage (Okagaki et al., 2015) (Table 5.1). There are currently no published genomes for *Phialophora* species within the *Gaeumannomyces-Phialophora* complex. The lack of genome sequence data makes it extremely difficult to identify species-specific genes or unique regions that could be utilised to design primers to distinguish between species within the *Gaeumannomyces-Phialophora* complex.

This PhD study reports on the isolation and characterisation of a *Phialophora* isolate collection from fields on the Rothamsted Farm to use for further analysis throughout this chapter as well as allowing pot bioassays to be performed to screen the ability of *Phialophora* spp. to colonise the roots of different cereal genotypes (Chapter 6). Pathogenicity tests were carried out to confirm the ability of the field isolates to colonise wheat roots, along with a PCR assay to differentiate the species identity through the use of ITS primers ITS5 and ITS4, allowing the fungal ribosomal DNA (rDNA) regions to be amplified in order to characterise the fungal species (White et al., 1990). The ITS regions of the fungal rDNA are highly variable and are a useful tool in distinguishing amongst closely related species. The two *Phialophora* species, *Phialophora* sp. lobed hyphopodia and the unnamed *Phialophora* sp. (see Ward and Bateman (1999) and Ulrich et al. (2000)) were successfully isolated from the Rothamsted Farm, whilst the *P. graminicola* species was sub-cultured from a cold room stock of an isolate previously collected from the Rothamsted Farm (Ward and Gray, 1992). This allowed the three *Phialophora* species to be sent for next-generation genome sequencing. This chapter reports on the first draft genome sequences of the three *Phialophora* species. The three draft genomes were subsequently annotated and comparative genomics were carried out with the aim of determining genes or regions of uniqueness in each of the three *Phialophora* genomes for future species-specific diagnostic assays.

Table 5.1. Genome and assembly summary statistics of the fungal species within the Broad's Institute *Magnaporthe* comparative database.

| **Genome statistics** | ***Magnaporthe* comparative species** | | |
|---|---|---|---|
| | ***Gaeumannomyces graminis* var. *tritici*** **Isolate: R3-111a-1** **Origin:** Wheat (Take-all decline field), Moses Lake, Washington, USA, 1980[1] | ***Magnaporthe oryzae*** **Strain: 70-15 (MG8)** **Origin:** Laboratory strain, 1991[2] | ***Magnaporthe poae*** **Strain: ATCC 64411** **Origin:** Kentucky bluegrass and annual blue grass, Northeastern and Midwestern USA[3] |
| Size | 43.62 Mb | 41.03 Mb | 39.5 Mb |
| Number of chromosomes | N/A | 7 | N/A |
| G + C (%) content | 56.85 | 51.61 | 56.99 |
| Number of predicted protein-coding genes | 14,463 | 12,827 | 12,169 |
| Number of predicted tRNA genes | 273 | 325 | 167 |
| Number of predicted rRNA genes | 13 | 40 | 29 |
| **Assembly statistics** | | | |
| Coverage | 46X | 7 chromosomes | 25X |
| Total contig length | 40.88 Mb | 40.95 Mb | 34.54 Mb |
| Number of scaffolds | 513 | 8 | 205 |
| Scaffold N50 (length of scaffold containing middle base of genome) | 6.7 Mb | 6.61 Mb | 3.43 Mb |
| Number of contigs | 1,808 | 219 | 3,106 |
| Contig N50 (length of contig containing middle base of genome) | 48.94 Kb | 823.59 Kb | 16.56 Kb |
| %Q40 (% of contig bases with quality score equal or greater than 40) | 99.63 | | 99.34 |

[1] Mazzola et al. (1995).

[2] Chao and Ellingboe (1991).

[3] Landschoot and Jackson (1989).

## 5.2 Materials and Method

### 5.2.1 Isolation of *Phialophora* spp. from root tissue

Five fields were sampled across the Rothamsted Farm during 2013 and one field during 2014 (Table 5.2). Fields were chosen due to *Phialophora* populations being found in previous field trials from 2009-2012. Soil cores were baited with 10 seeds (variety Hereward) and placed in a controlled environment room for 5 weeks (16 hour day, 70% relative humidity, day/night temperatures 15/10°C, 250 µmol light, twice weekly watering). Roots were washed free of soil and assessed for the characteristic symptoms of runner hyphae and sub-epidermal vesicles of *Phialophora* infection. *Phialophora* spp. were then isolated from colonised root pieces as described in Chapter 2: General Materials and Methods. A total of 22 isolates were obtained.

DNA was extracted from each of the 22 field isolates, PCR was performed to amplify the internal transcribed spacers (ITS) 5 and ITS 4 region and sequences were sent for sequencing analysis, methodology described in Chapter 2: General Materials and Methods.

There was a problem with bacterial contamination from a number of *Phialophora* root isolations. A bacterial 16S rRNA gene PCR assay was carried out to identify the bacterial species from three contaminated PDA plates. A crude DNA extraction was performed from the three plates by firstly selecting and removing a colony with a sterile toothpick and placing into and Eppendorf with 100 µl of sterile distilled $H_2O$ for each plate. The samples were then vortexed and placed in a heat block at 95°C for 5 mins to lyse the cells. The cell preparation was then spun down in a centrifuge at 14,000 rpm.

A PCR assay was then performed using a thermal cycler (G-STORM GS1) with the primers MuyzerF (CCTACGGGAGGCAGCAG) (Muyzer et al., 1993) and 1389R (ACGGGCGGTGTGTACAAG) (Osborn et al., 2000) to amplify the variable regions V3 to V8 of the 16S rRNA genes found in bacteria, in order to identify the bacterial species contaminating the *Phialophora* root isolations. A 25 µl reaction was set up in a flow hood, to prevent bacterial contamination, and contained 2.5 µl of Bioline

PCR buffer (Bioline, London, UK), 0.75 µl $MgCl_2$ (2Mm), 1 µl of each primer (1 µM) (synthesised by Eurofins MWG Operon), 0.5 µl dNTP (200 µM), 0.25 µl Bioline *Taq* polymerase (1.25 U) and 1 µl of cell lysate or 1 µl of sterile distilled $H_2O$ for the negative control. Amplification conditions consisted of an initialisation step at 95°C for 15 min followed by 35 cycles with a denaturation step at 95°C for 30 secs, an annealing step at 56°C for 30 secs, an extension step at 72°C for 1 min, and a final extension step at 72°C for 10 min and final hold at 10°C. PCR products (20 µl) were separated on a 1.2% agarose gel electrophoresis (see Chapter 2: General Materials and Methods) at 80 V for 1 hour. The gel was visualised using ethidium bromide staining and photographed under UV light (see Chapter 2: General Materials and Methods) and the bands were excised and purified using a Wizard® SV Gel and PCR Clean-up System kit (Promega, USA) as per the manufacturer's instructions. The PCR products were quantified using the Nanodrop ND-1000 Spectrophotometer and sent to Eurofins MWG Operon for sequencing analysis and returned sequences were entered into the NCBI BLAST database for species identification.

### 5.2.2 Colonisation assay of field isolates

A pot bioassay was set-up to test the wheat root colonisation ability of the 22 field isolates. A randomised block design was calculated in GenStat (VSNI, Hemel Hempstead, UK) and included three replicates for each of the isolates and three replicates of an experimental negative control (variety Hereward). Plastic cups (7.5 cm diameter x 11 cm tall, drilled with 4 drainage holes, 3 mm diameter) were filled with 250 g of damp coarse soil. A third was cut from a confluent colonised PDA plate for each isolate and macerated with 50 $cm^3$ of damp coarse sand and added to the pots. A third of a non-colonised PDA plate was used for the three negative control pots. A 100 $cm^3$ layer of damp coarse sand was then poured on top and 5 seeds (variety Hereward) were placed on the surface and covered with a layer of horticultural grit. Pots were placed in a controlled environment room (16 hour day, 70% relative humidity, 15°C day/10°C night, twice weekly watering) for 5 weeks. After 5 weeks the roots were washed free of soil and immersed in water in a white dish to visually examine and record the number of sub-epidermal vesicles per root.

The total number of plants and roots and the number of *Phialophora* infected plants and roots were recorded in order to calculate percentages.

### 5.2.3 Isolation of *Phialophora graminicola* from cold room water stocks

A field isolate of *P. graminicola* was not obtained from the field isolations across the Rothamsted Farm. *Phialophora graminicola* isolates were therefore sub-cultured from cold room universal bottle water stocks stored at RRes (Table 5.3) (Ward and Gray, 1992). Three *P. graminicola* isolates were chosen from the isolate stocks at RRes, in order to increase the chance of successfully sub-culturing an isolate. A 1 $cm^2$ agar plug was removed from each of the four water stocks (Table 5.3) and placed onto PDA amended with penicillin (50 µg per PDA plate) and streptomycin (50 µg per PDA plate) in a sterile flow hood. Plates were incubated at 15°C for ~ two weeks. Cultures were transferred using a sterile tooth pick onto fresh PDA amended with penicillin (50 µg per PDA plate) and streptomycin (50 µg per PDA plate) and incubated at 15°C for ~ two weeks. This method was repeated a number of times if plates continued to be contaminated. Problems with bacterial contamination meant that only isolate 74/1736-2 (20.03.91) (Table 5.3) was taken forward for future work.

Gail Canning set up a pathogenicity assay to test whether the *P. graminicola* 74/1736-2 (20.03.91) isolate (Table 5.3) could colonise wheat roots or whether the isolate had lost the ability to colonise the roots whilst stored in sterile water for the past 24 years. Three replicates of the 74/1736-2 isolate were set up in plastic cups, washed and assessed as detailed in section 5.2.2.

Table 5.2. Details of sampled fields across the Rothamsted Farm for collecting *Phialophora* isolates.

| Rothamsted field | Sample date | Number of sampled cores | Crop and variety (var.) | Number of isolates obtained |
|---|---|---|---|---|
| Fosters | 27.06.13 | 45 | Winter wheat stubble, var. Conqueror and spring barley stubble, var. Tipple | 1 |
| Great Knott III | 12.08.13 | 90 | Winter barley stubble, var. Saffron | 6 |
| New Zealand | 28.08.13 | 50 | Spring barley stubble, var. Tipple | 6 |
| Pastures | 03.09.13 | 100 | Winter wheat stubble, var. Conqueror | 6 |
| Summerdells I | 03.09.14 | 100 | Winter wheat stubble, var. Conqueror | 3 |

Table 5.3. Details of the *Phialophora graminicola* isolates from the cold room water stocks at Rothamsted Research (RRes).

| ***Phialophora* species**[1] | **Code**[1] | **Original host**[1] | **Place and year of origin**[1] | **Isolated by**[1] | **Set-up date of water stock** |
|---|---|---|---|---|---|
| *Phialophora graminicola* | 89/2-1B | Wheat | West Barnfield, RRes, 1985 | R. J. Gutteridge | 20.03.91 |
| *Phialophora graminicola* | 76/73-2 | Wheat | Garden plot V, RRes, 1976 | R. J. Gutteridge | 19.02.92 |
| *Phialophora graminicola*[2] | 74/1736-2 | Ryegrass | Highfield[3], RRes, 1974 | R. J. Gutteridge | 20.03.91 |
| *Phialophora graminicola* | 74/1736-2 | Ryegrass | Highfield[3], RRes, 1974 | R. J. Gutteridge | 15.07.91 |

[1] Details obtained from Ward and Gray (1992).

[2] Isolate taken forward for next-generation sequencing.

[3] The Highfield experiment is a long-term field experiment, started in 1949, at RRes. The Highfield site was in permanent grass for a number of centuries, some plots stayed in permanent grass, whilst others went into continuous arable and others alternated between arable and ley. This provides a valuable resource of the effects of cropping practices on the soil organic matter and crop yield.

### 5.2.4 DNA extractions of three *Phialophora* species for next-generation sequencing

Three *Phialophora* species and isolates were chosen for next-generation sequencing; the *P. graminicola* RRes cold room isolate, the unnamed *Phialophora* sp. isolated from Summerdells I field on Rothamsted Farm and a *Phialophora* sp. lobed hyphopodia isolated from the same field that the field trial in Chapter 6 was carried out in, New Zealand field on the RRes Farm (Table 5.4). A 1 $cm^3$ agar plug was cut from the stored *Phialophora* PDA plates and transferred to a fresh PDA or Czapek Dox agar (45.4 g in 1 litre of distilled water, autoclave) plate (Table 5.4), amended with penicillin (50 µg) and streptomycin (50 µg), in a sterile air flow hood. The plates were incubated at 15°C until confluent, ~ two weeks. A 1 $cm^3$ agar plug was then cut from the confluent plates of each of the three *Phialophora* isolates (Table 5.4) and transferred to a 250 ml conical flask containing 50 ml PDB (Potato Dextrose Broth) amended with penicillin/streptomycin (100 ng/ml). Cultures were incubated at 21°C on an orbital shaker at 250 rpm for 5 days. The cultures were macerated using a sterile Waring blender for 30 seconds. In a sterile air flowhood, 250 ml PDB amended with penicillin/streptomycin (100 ng/ml) was transferred into 1500 ml conical flasks and 50 ml of the macerated culture was added. Cultures were incubated at room temperature on an orbital shaker at 180 rpm for 7 days. Cultures were filtrated through a layer of Miracloth using a vacuum pump and washed three times with sterile distilled $H_2O$ before drying. Mycelium was transferred into 2 ml Eppendorfs and freeze-dried for 48 hrs and then ground in a mortar and pestle under liquid nitrogen and transferred into a 50 ml tube and stored at -20°C.

DNA was extracted using a method previously modified by Martin Urban at RRes from Xu and Leslie (1996). The protocol was further modified as several attempts of extraction were required to pass quality control checks at the Earlham Institute (EI) (previously The Genome Analysis Centre (TGAC)) (EI, Norwich Research Park, Norwich, UK). A 50 ml tube was filled with 2 ml of ground mycelium. DNA was extracted with 15/20 ml CTAB (cetyltrimethylammonium bromide) lysis buffer (see Appendix 5.1). Samples were mixed before the addition of β-mercaptoethanol (100

µl β-mercaptoethanol/20 ml) and RNase ([10 µg/µl]/20 ml). Samples were incubated at 65°C in a water bath for 30 mins and inverted every 10 mins before cooling on ice. One volume of CIA (chloroform:isoamyl alcohol) (24:1) (see Appendix 5.1) was added and tubes were placed on a shaking platform for 15 mins before centrifugation in a swing-out centrifuge at 3,400 *g* at 4°C for 5 mins. The upper aqueous phase was transferred into a fresh 50 ml tube with the addition of one volume of isopropanol. DNA was precipitated by centrifugation in 30 ml Corex glass tubes at 12,000 *g* at 15°C for 10 mins. The pellet was washed with 5 ml of 70% sterile ethanol. Ethanol was discarded and pellets were air-dried before resuspending in sterile distilled $H_2O$ and transferring to 2 ml Eppendorf tubes.

A further extraction step was performed on *Phialophora* sp. lobed hyphopodia and *Phialophora graminicola* isolate submissions in January 2015 and *Phialophora* sp. lobed hyphopodia isolate submissions in May 2015 (Table 5.5) with the addition of 1 ml phenol:chloroform:isoamyl alcohol (25:24:1). Tubes were inverted before centrifugation at 16,000 *g* at room temperature for 5 mins and then placed on ice for 5 mins. The upper aqueous phase was transferred into fresh 2 ml Eppendorf tubes. An additional phenyl/chloroform extraction was carried out by transferring 1 ml phenyl/chloroform and inverting tubes before centrifugation at 13,000 rpm at room temperature for 5 mins and then placing on ice for 5 mins. A further 1 ml of chloroform (topped up with TE to increase the upper phase layer) was added and tubes were inverted several times before centrifugation at 16,000 *g* for 5 min. The upper layer was then transferred into a fresh 2 ml Eppendorf tube. DNA was precipitated by transferring one volume of isopropanol and inverting the tube several times before leaving at room-temperature for 15 mins. Tubes were then centrifuged at 16,000 *g* for 10 mins and the isopropanol was removed. The pellet was washed with 400 µl of 4°C ethanol and the tube was inverted several times before centrifugation at 16,000 *g* for 5 min. Ethanol was removed and the pellet was air-dried at room temperature for 5 min before resuspending in 400 µl sterile $H_2O$ and storing at -20°C.

DNA was purified using Qiagen Plasmid or Cosmid DNA Purification Kit and following the Plasmid Midi Kit protocol from step 9. A Qiagen-tip 100 was

equilibrated by applying 4 ml buffer QBT and 8 ml/12 ml of QBT buffer and 600 µl/800 µl DNA was transferred to a 15 ml tube and the contents was poured into the Qiagen-tip. A vacuum pump was used throughout the protocol due to the filter in the Qiagen-tip becoming blocked. The Qiagen-tip was washed with 2 x 10 ml of Buffer QC. DNA was eluted into a 50 ml tube with 5 ml of Buffer QF. DNA was precipitated by adding 8 ml of 100% room-temperature ethanol and gently mixing before transferring into 15 ml corex glass tubes and centrifuging at 14,600 *g* at 4°C for 20 mins. DNA pellet was washed with 2 ml of 70% room-temperature ethanol and centrifuged at 14,600 *g* for 10 mins. The pellet was air-dried for 5 -10 mins and resuspended in 50 µl $H_2O$ and stored at -20°C. Extracted DNA was quantified using the Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA) or the Qubit (Thermo Fisher Scientific, USA) and stored at -20°C (Table 5.5).

The Plasmid Midi Kit protocol was modified for the January/May DNA extractions as follows. A Qiagen-tip 500 was equilibrated by applying 10 ml buffer QBT and of 6 ml of QBT buffer and 400 µl DNA DNA was transferred to a 15 ml tube and the contents was poured into the Qiagen-tip. The Qiagen-tip was washed with 2 x 30 ml of Buffer QC and transferred to a 50 ml tube to elute DNA with 15 ml of Buffer QF. DNA was eluted into a 50 ml tube with 15 ml Buffer QF. DNA was then precipitated by adding 30 ml of 100% ethanol and gently mixing the tubes before transferring into a 15 ml and 30 ml Corex glass tube and centrifuging at 14,600 *g* at 4°C for 30 mins. DNA pellet was washed with 5 ml of 70% room-temperature ethanol and centrifuged at 14,600 *g* for 10 mins. An additional centrifugation step was carried out due to the lack of a visible pellet. The pellet was then air dried for 5-10 mins and resuspended in 50-100 µl $H_2O$ and stored at -20°C. Extracted DNA was quantified using the Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, USA) or the Qubit (Thermo Fisher Scientific, USA) and stored at -20°C (Table 5.5).

Table 5.4. Details of the three *Phialophora* species chosen for *de novo* next-generation sequencing at the Earlham Institute.

| ***Phialophora* species** | **Isolate ID** | **Original host** | **Place and year of origin** | **Media that isolate was grown on** |
|---|---|---|---|---|
| *Phialophora graminicola* | 74/1736-2 | Ryegrass | Highfield, RRes, 1974[1] | Potato dextrose agar |
| *Phialophora* sp. lobed hyphopodia | N.14.13 | Wheat | New Zealand, RRes, 2013 | Czapek dox agar |
| *Phialophora sp.* | S.09.13 | Wheat | Summerdells I, RRes, 2013 | Potato dextrose agar |

[1] Ward and Gray (1992).

Table 5.5. DNA submission details of the three *Phialophora* species sent for *de novo* next-generation sequencing at the Earlham Institute.

| **Date sequenced** | ***Phialophora* species** | **Concentration (ng/µl)** | **Sample volume (µl)** | **Quantification** | **260/280 ratio** | **260/230 ratio** | **Buffer** | **Kit** |
|---|---|---|---|---|---|---|---|---|
| Dec 2014 | *Phialophora graminicola* | 100 | 78 | Nanodrop | 1.89 | 1.94 | water | Qiagen |
| Dec 2014 | *Phialophora* sp. lobed hyphopodia | 100 | 78 | Nanodrop | 1.89 | 2.14 | water | Qiagen |
| Dec 2014[1] | *Phialophora* sp. | 100 | 78 | Nanodrop | 1.90 | 1.95 | water | Qiagen |
| March 2015 | *Phialophora* sp. lobed hyphopodia | 100 | 219 | Nanodrop | 1.90 | 2.00 | water | Qiagen |
| March 2015[1] | *Phialophora graminicola* | 100 | 400 | Nanodrop | 1.89 | 2.10 | water | Qiagen |
| June 2015[12] | *Phialophora* sp. lobed hyphopodia | 185 | 70 | Qubit | 1.90 | 2.11 | water | Qiagen |
| June 2015 | *Phialophora* sp. lobed hyphopodia | 44 | 70 | Nanodrop | 1.76 | 2.10 | water | Qiagen |

[1] DNA sample that was successfully sequenced.

[2] DNA samples previously extracted and sequenced March 2015 and then sent as a repeat submission and sequenced in June 2015.

### 5.2.5 *De novo* genome sequencing

The extracted *Phialophora* spp. DNA was sent to the EI (Norwich, UK) for *de novo* next-generation sequencing. The *Phialophora* spp. DNA samples were sent in three batches due to previous DNA samples failing the quality control checks at the EI. The *Phialophora sp*. was successfully sequenced in the first batch, the *P. graminicola* isolate was successfully sequenced in the second batch and the *Phialophora* sp. lobed hyphopodia successfully sequenced in the third and final batch (Table 5.5). Illumina sequencing was performed by the EI. The Illumina HiSeq 2500 sequencing platform was used to generate 125 bp paired-end reads.

### 5.2.6 *De novo* genome assembly and annotation

RRes bioinformatician Dr Rob King *de novo* assembled the sequence data without pre-processing by SOAPdenovo 2 (v2.04) (Luo et al., 2012) combining a range of k-mer values from 61-125, other parameters were left as default. The genomes were then annotated in Geneious (Biomatters Ltd., v8.1.3). The AUGUSTUS software plugin (Stanke and Morgenstern, 2005) for Geneious (Biomatters Ltd., v8.1.3) was used as a eukaryotic gene prediction program for each of the three *Phialophora* genomes. The species-specific parameter was set to the related rice blast species *Magnaporthe oryzae* (formerly *M. grisea*), since *Phialophora* species of the *Gaeumannomyces-Phialophora* complex are within the family *Magnaporthaceae.*

### 5.2.7 Genome comparisons

In order to identify regions or genes of uniqueness to each of the three *Phialophora* draft genomes, the annotated *Ggt* R3-111a-1 genome (assembly: Gae_graminis_V2, International Nucleotide Sequence Database Collaboration (INSDC) assembly GCA_000145635.1) was downloaded from Ensembl Fungi (EMBL-EBI). The ITS5-ITS4 rDNA sequences were extracted from each of the *Phialophora* draft genomes and aligned with the region from the downloaded *Ggt* R3-111a-1 genome in Geneious (Biomatters Ltd., v8.1.3).

Genome completeness was assessed for the three *Phialophora* draft genome sequences as well as the downloaded annotated *Ggt* R3-111a-1 genome (Ensembl Fungi, EMBL-EBI) with the Benchmarking Universal Single-Copy Orthologs (BUSCO)

(Waterhouse et al., 2013) v1.1b1 pipeline (Simao et al., 2015) using the 1,438 core fungal genes against the genome sequences using the pre-computed Augustus metaparameters from *Fusarium graminearum* and the full optimisation option for Augustus gene finding training. Simao et al. (2015) used the OrthoDB database of orthologs (www.orthodb.org) to characterise different phylogenetic clades. There are currently BUSCO profiles for the phylogenetic clades of arthropods, vertebrates, fungi, bacteria, metazoans, eukaryotes and early access is available for a plants BUSCO profile (http://busco.ezlab.org/). The BUSCO analysis was then used to identify whether any of the core genes that were complete in the *Phialophora* genome sequences were missing from the *Ggt* genome and could therefore be targeted as regions of uniqueness. Venn diagrams were constructed to illustrate the number of complete BUSCOs from different combinations of the three *Phialophora* draft genomes and the *Ggt* R3-111a-1 genome (Ensembl Fungi, EMBL-EBI). The OrthoDB7 2013 database (http://cegg.unige.ch/orthodb7) was used to provide descriptions of proteins and gene ontologies from identified BUSCOs within the BUSCO analysis. The OrthoDB7 2013 database contains a collection of orthologous protein-coding gene from across 64 vertebrates, 57 arthropods, 175 fungi, 14 basal metazoans, and 1115 bacteria (http://cegg.unige.ch/orthodb7).

In order to perform a BLAST protein call of each species against the three remaining species, the coding sequence (CDS) was extracted from each of the annotated *Phialophora* draft genomes, as well as the CDS from the downloaded *Ggt* R3-111a-1 genome, and translated to proteins to obtain a predicted proteome of each species in Geneious (Biomatters Ltd., v8.1.3). In order to ascertain what predicted gene coding regions were unique to each of the four proteomes, three of the four proteomes were merged together in turn and a custom BLAST database was created for each of the three sets of proteomes. The remaining proteome was then blasted against the custom database, with the max hit set to 1, and the E-value set to 1e-1 and the BLAST databases were exported into Microsoft Excel (Microsoft Corporation, USA). The gene sequence names were extracted from each of the individual *Phialophora* draft genomes and exported into Microsoft Excel (Microsoft Corporation, USA). The VLOOKUP function was used to identify which proteins were

absent from the individual *Phialophora* species as well as the percentage of identical sites from proteins presence in all four fungal species.

## 5.3 Results

### 5.3.1 Pathogenicity test and ITS sequencing of the *Phialophora* field isolate collection

The 22 field isolates recovered from soil post-harvest of a wheat crop in 2013 were tested for wheat colonisation. Thirteen of the isolates infected the wheat seedling roots through the production of fungal runner hyphae. Nine of the isolates were visually identified as *Phialophora* species due to the presence of sub-epidermal vesicles and four as the take-all fungus due to the production of black necrotic lesions (Table 5.6). Nine isolates were visually unidentified due to the lack of the characteristic fungal runner hyphae and sub-epidermal vesicles of *Phialophora* colonisation. Two *Phialophora* species were visually identified: eight *Phialophora* sp. lobed hyphopodia isolates due to the presence of large single sub-epidermal vesicles Figure 5.1) and one isolate initially thought to be *P. graminicola* due to the presence of small clustered sub-epidermal vesicles (Figure 5.2) (Table 5.6).

The DNA was then extracted from the 22 field isolates and a PCR assay was carried out to amplify the ITS regions (Figure 5.3). Following amplification, the PCR products were sent for sequencing and the returned sequences were blasted in NCBI for species identification and to confirm the initial visual identifications of the pathogenicity assay. Nine isolates were confirmed as *Phialophora* species (Table 5.6). Visual identification matched with the species identification for all but one *Phialophora* species. Isolate S.09.13 was identified as a *Phialophora* sp. rather than the visual identification of *P. graminicola* (Table 5.6). All of the sampled fields provided a *Phialophora* isolate, except for Fosters and Great Knott III. The four isolates G.33.13, G.41.13, G.42.13 and S.08.13 were confirmed to be the take-all fungus *Ggt* (Table 5.6). The remaining nine isolates that did not cause *Phialophora*-like infections were identified to be other fungal, oomycete or yeast species (Table 5.6).

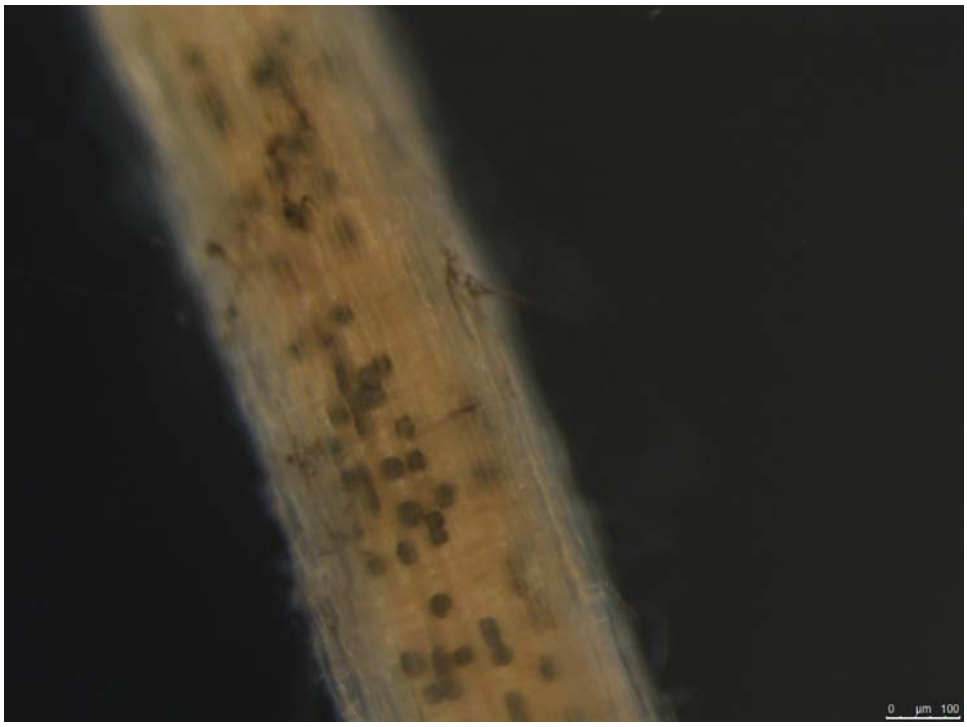



**Figure 5.1.** Microscopy image of *Phialophora* sp. lobed hyphopodia, field isolate P.10.13, colonised wheat seedling root. Magnification X 111.

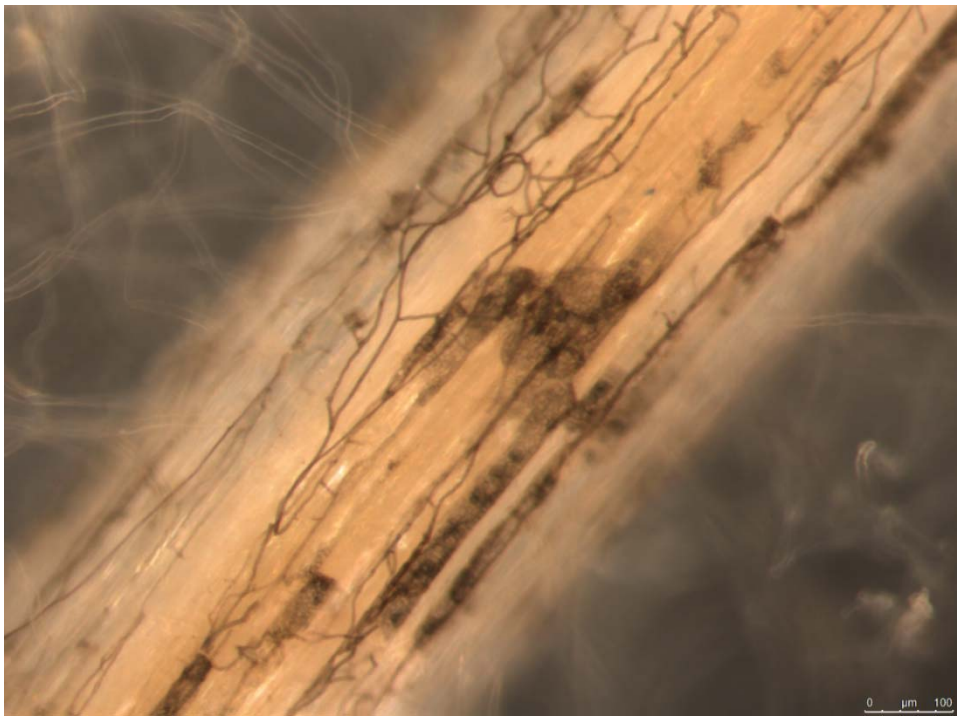



**Figure 5.2.** Microscopy image of *Phialophora* sp., field isolate S.09.13, colonised wheat seedling root. Magnification X 134.

Table 5.6. Identification of the 22 field isolates through visual morphological identification and ITS sequencing results.

| Isolate ID | Rothamsted field | Visual identification | NCBI top BLAST search |
|---|---|---|---|
| F.01.13 | Fosters | No *Phialophora* colonisation | Uncultured soil fungus/*Ilyonectria* sp. |
| N.01.13 | New Zealand | No *Phialophora* colonisation | *Debaryomyces hansenii* (99%) |
| N.14.13[1] | New Zealand | *P*. sp. lobed hyphopodia | *P*. sp. lobed hyphopodia (99%) Wheat, RRes, Hornby (Bryan et al., 1995) |
| N.15.13 | New Zealand | No *Phialophora* colonisation | *Microdochium nivale/Monographella nivalis* (100%) |
| N.16.13 | New Zealand | No *Phialophora* colonisation | *Mucor hiemalis* (99%) |
| N.17.13 | New Zealand | No *Phialophora* colonisation | *Microdochium nivale/Monographella nivalis* (99%) |
| N.20.13 | New Zealand | *P*. sp. lobed hyphopodia | *P*. sp. lobed hyphopodia (99%) Wheat, RRes, Hornby (Bryan et al., 1995) |
| G.32.13 | Great Knott III | No *Phialophora* colonisation | *Microdochium nivale/Monographella nivalis* (100%) |
| G.33.13 | Great Knott III | *Ggt* | *Ggt* (99%) |
| G.35.13 | Great Knott III | No *Phialophora* colonisation | *Pythium sylvaticum* (100%) |
| G.38.13 | Great Knott III | No *Phialophora* colonisation | *Microdochium nivale/Monographella nivalis* (99%) |
| G.41.13 | Great Knott III | *Ggt* | *Ggt* (99%) |
| G.42.13 | Great Knott III | *Ggt* | *Ggt* (99%) |
| S.03.13 | Summerdells I | *P*. sp. lobed hyphopodia | *P*. sp. lobed hyphopodia (99%) Wheat, RRes, Hornby (Bryan et al., 1995) |
| S.08.13 | Summerdells I | *Ggt* | *Ggt* (99%) |
| S.09.13[1] | Summerdells I | *P. graminicola* | *Phialophora* sp. (99%) Wheat, Germany (Ulrich et al., 2000)/ Wheat, RRes (Ward and Bateman, 1999) |
| P.03.13 | Pastures | *P*. sp. lobed hyphopodia | *P*. sp. lobed hyphopodia (99%) Wheat, RRes, Hornby (Bryan et al., 1995) |
| P.05.13 | Pastures | *P*. sp. lobed hyphopodia | *P*. sp. lobed hyphopodia (99%) Wheat, RRes, Hornby (Bryan et al., 1995) |
| P.06.13 | Pastures | *P*. sp. lobed hyphopodia | *P*. sp. lobed hyphopodia (99%) Wheat, RRes, Hornby (Bryan et al., 1995) |
| P.08.13 | Pastures | No *Phialophora* colonisation | *Microdochium nivale/Monographella nivalis* (99%) |
| P.09.13 | Pastures | *P*. sp. lobed hyphopodia | *P*. sp. lobed hyphopodia (99%) Wheat, RRes, Hornby (Bryan et al., 1995) |
| P.10.13 | Pastures | *P*. sp. lobed hyphopodia | *P*. sp. lobed hyphopodia (99%) Wheat, RRes, Hornby (Bryan et al., 1995) |

[1] *Phialophora* spp. field isolates sent to the Earlham Institute for *de novo* next-generation sequencing.

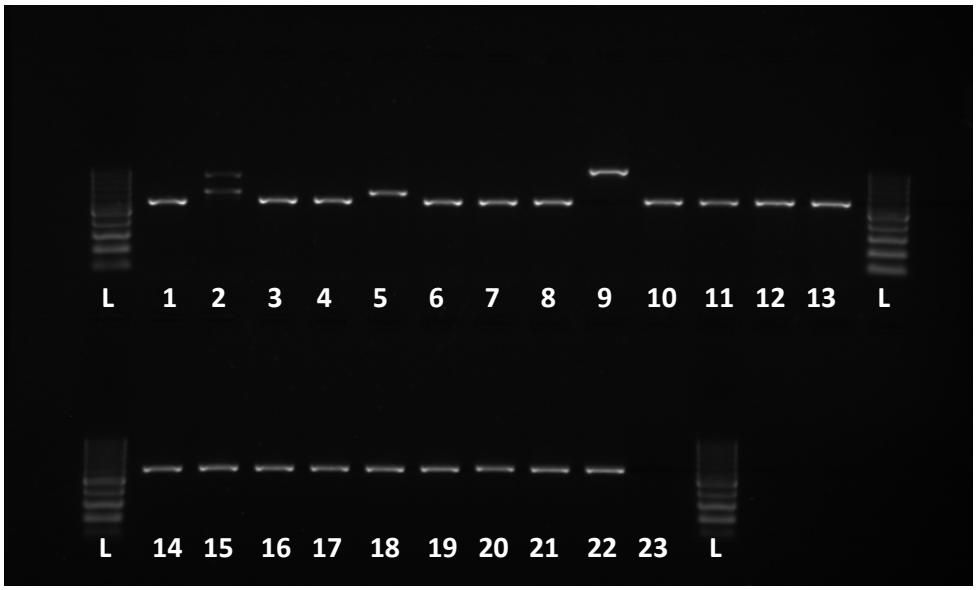



Figure 5.3. ITS PCR analysis of 22 field isolates (lanes 1-22) obtained from wheat seedling root isolations of baited soil cores using primers ITS5F and ITS4R. Lane L: 100 bp DNA ladder (Thermo Fisher Scientific, USA), lane 1: F.01.13, lane 2: N.01.13, lane 3: N.14.13, lane 4: N.15.13, lane 5: N.16.13, lane 6: N.17.13, lane 7: N.20.13, lane 8: G.32.13, lane 9: G.35.13, lane 10: G.33.13, lane 11: G.38.13, lane 12: G.41.13, lane 13: G.42.13, lane 14: S.03.13, lane 15: S.08.13, lane 16: S.09.13, lane 17: P.03.13, lane 18: P.05.13, lane 19: P.06.13, lane 20: P.08.13, lane 21: P.09.13, lane 22: P.10.13 and lane 23: negative control, no DNA.

Bacterial contamination was often found when isolating *Phialophora* fungi from root tissue. The bacterial contamination was consistent in appearance and DNA was extracted from three contaminated field isolates and a bacterial 16S rRNA PCR assay was carried out to identify the bacterial species. A BLAST analysis of the returned sequences revealed that the three bacterial isolates were *Pseudomonas* spp., species-specific *Pseudomonas* primers would have to be used in order to identify the isolates to the species level. The three *Phialophora*-like field isolates could not be salvaged from the *Pseudomonas* spp. contamination.

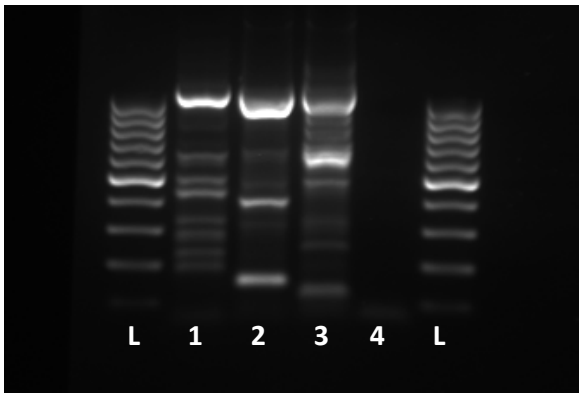



Figure 5.4. 16S rRNA PCR analysis from three contaminated *Phialophora* isolates (lanes 1-3) using primers MuyzerF and 1389R. Lane L: 100 bp DNA ladder (Thermo Fisher Scientific, USA), lane 1: S.04.13, lane 2: S.06.13, lane 3: N.13.13 and lane 4: negative control, no DNA.

Unfortunately, *P. graminicola* was not isolated from the field and the species was sub-cultured from a cold room stock stored at RRes in order to complete the *Phialophora* isolate collection. The cold room stock of *P. graminicola* had the ability to colonise the wheat seedling roots, even after 24 years of being stored in sterile water (Figure 5.5). The complete *Phialophora* isolate collection therefore consisted of the cold room stock of *P. graminicola* and the nine *Phialophora* field isolates, comprised of one unnamed *Phialophora* sp. and eight *Phialophora* sp. lobed hyphopodia species (Table 5.7).

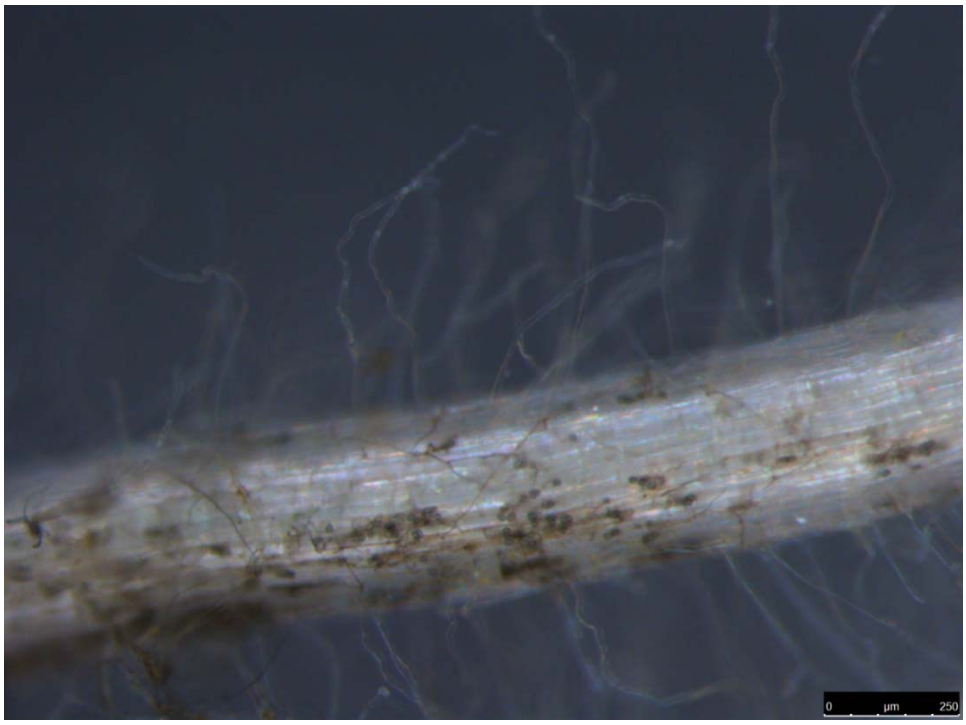



Figure 5.5. Microscopy image of *Phialophora graminicola*, cold room stock 74/1736-2 (20.03.91) (Ward and Gray, 1992), colonised wheat seedling root. Magnification X 80.8.

Table 5.7. Complete *Phialophora* isolate collection.

| **Isolate ID** | **Original host** | **Rothamsted field and year of origin** | ***Phialophora* species** |
|---|---|---|---|
| N.14.13 | Wheat | New Zealand, RRes, 2013 | *Phialophora* sp. lobed hyphopodia |
| N.20.13 | Wheat | New Zealand, RRes, 2013 | *Phialophora* sp. lobed hyphopodia |
| S.03.13 | Wheat | Summerdells I, RRes, 2013 | *Phialophora* sp. lobed hyphopodia |
| S.09.13 | Wheat | Summerdells I, RRes, 2013 | *Phialophora* sp. |
| P.03.13 | Wheat | Pastures, RRes, 2013 | *Phialophora* sp. lobed hyphopodia |
| P.05.13 | Wheat | Pastures, RRes, 2013 | *Phialophora* sp. lobed hyphopodia |
| P.06.13 | Wheat | Pastures, RRes, 2013 | *Phialophora* sp. lobed hyphopodia |
| P.09.13 | Wheat | Pastures, RRes, 2013 | *Phialophora* sp. lobed hyphopodia |
| P.10.13 | Wheat | Pastures, RRes, 2013 | *Phialophora* sp. lobed hyphopodia |
| 74/1736-2[1] | Ryegrass | Highfield, RRes, 1974 | *Phialophora graminicola* |

[1] Ward and Gray (1992).

### 5.3.2 Genome comparisons

Major genome differences were found across the three *Phialophora* draft genomes. Firstly, there was a noticeable difference in the number of reads obtained. *Phialophora graminicola* had the largest number of reads, 25,719,585, whilst *Phialophora* sp. and *Phialophora* sp. lobed hyphopodia had a smaller number of reads, 14,670,529 and 13,197,250 respectively (Table 5.8). The G + C content was found to be consistent across all three *Phialophora* species.

Although *P. graminicola* had a smaller average insert size than the two other *Phialophora* genomes, the *P. graminicola* draft genome had the smallest number of contigs and scaffolds, the greater mean contig and scaffold size, as well as the largest contig and scaffold size (Table 5.8). An estimated genome coverage was calculated, using the *Ggt* R3-111a-1 genome size as an estimation of the *Phialophora* genome size. As expected the *P. graminicola* draft genome had a considerably higher genome coverage than *Phialophora* sp. lobed hyphopodia and *Phialophora* sp. (Table 5.8). The genome coverage of *Phialophora* sp. lobed hyphopodia and *Phialophora* sp. were similar, however there was a noticeable difference in their assembly. The *Phialophora* sp. draft genome had a higher number of contigs and scaffolds and a lower mean contig and scaffold size. The largest scaffold size of the *Phialophora* sp. draft genome was less than the largest scaffold found in both *Phialophora* sp. lobed hyphopodia and *Phialophora* sp., nevertheless the scaffold had the highest number of predicted genes (Table 5.8).

Table 5.8. Genome assembly summary statistics of the three *Phialophora* draft genome sequences.

| **Genome summary statistics** | ***Phialophora* species** | | |
|---|---|---|---|
| | ***Phialophora* sp. lobed hyphopodia**<br>**Isolate:** N.14.13<br>**Field:** New Zealand, RRes<br>**Year:** 2013<br>**Crop:** spring barley, var. Tipple | ***Phialophora* sp.**<br>**Isolate:** S.09.13<br>**Field:** Summerdells I, RRes<br>**Year:** 2013<br>**Crop:** wheat, var. Conqueror | ***Phialophora graminicola***<br>**Isolate:** 74/1736-2<br>**Field:** Highfield, RRes<br>**Year:** 1974[1]<br>**Crop:** Ryegrass[1] |
| Reads | 13,197,250 | 14,670,529 | 25,719,585 |
| G + C (%) content | 55.10% | 56.00% | 55.00% |
| Average insert size less adaptors | 538 | 513 | 439 |
| Max read length | 126 | 126 | 126 |
| Coverage | 38.67X | 42.99X | 75.36X |
| Number of contigs | 29,543 | 66,897 | 27,247 |
| Mean contig size | 1208 | 676 | 1408 |
| Largest contig size | 27,543 | 20,824 | 62,518 |
| Contig N50 | 2012 | 2533 | 2211 |
| Number of scaffolds | 23,700 | 52,425 | 20,638 |
| Mean size of scaffolds | 1513 | 899 | 1870 |
| Largest scaffold size (no. predicted genes) | 59,189 (2) | 50,720 (14) | 167,376 (1) |
| Scaffold N50 | 2844 | 5199 | 3295 |
| Number of contigs in scaffold | 9,954 | 19,606 | 10,997 |

[1] Ward and Gray (1992).

#### 5.3.2.1 Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis of the completeness of the three *Phialophora* draft genomes

The ITS5-ITS4 rDNA regions of the three *Phialophora* draft genomes were extracted and aligned (Figure 5.6). Unfortunately, only a very short 199 bp sequence was extracted from the unnamed *Phialophora* sp. draft genome, yet within this 199 bp region there were only six single nucleotide polymorphisms (SNPs) (Figure 5.6). The ITS5-ITS4 rDNA regions were too similar amongst the three *Phialophora* draft genomes and the *Ggt* R3-111a-1 genome and therefore a BUSCO analysis was carried out to identity potential differences in the number of core fungal genes.

The BUSCO v1.1b1 pipeline (Simao et al., 2015) was used to identify core fungal genes in the *Phialophora* assemblies as a measure of the completeness of the draft genomes and to compare these against the *Ggt* R3-111a-1 genome (Ensembl Fungi, EMBL-EBI). The BUSCO analysis identified that the three *Phialophora* draft genomes had considerably fewer complete BUSCOs (28%-52%), and therefore a low level of genome completeness than those identified for the *Ggt* genome (97%) (Table 5.9) (Figure 5.6). When comparing the level of completeness across the three *Phialophora* species, the *Phialophora* sp. draft genome had the most complete BUSCOs (52%), followed by only 30% complete BUSCOs for the *P. graminicola* draft genome and 28% complete BUSCOs for the *Phialophora* lobed hyphopodia draft genome (Table 5.9) (Figure 5.6). Of those complete BUSCOs, a fraction were duplicated; the complete duplicated BUSCOs are included in the count of the complete single-copy BUSCOs. A higher percentage of BUSCOs were fragmented across *P. graminicola* and *Phialophora* sp. lobed hyphopodia than the percentage of complete BUSCOs identified for these two species. A similar amount of missing BUSCOs, genes not recovered, to complete BUSCOs were found for the *P. graminicola* and *Phialophora* sp. lobed hyphopodia draft genomes, over double the amount of missing BUSCOs when compared to *Phialophora* sp. draft genome and a substantially higher amount of missing BUSCOs than found for the *Ggt* genome (Table 5.9).

**Figure 5.6.** ITS5-ITS4 rDNA sequences extracted from the three *Phialophora* draft genomes and aligned with the *Gaeumannomyces graminis* var. *tritici* (*Ggt*) R3-111a-1 annotated genome downloaded from Ensembl Fungi. Red box indicates the 199 bp alignment of all three *Phialophora* spp. and *Ggt*.

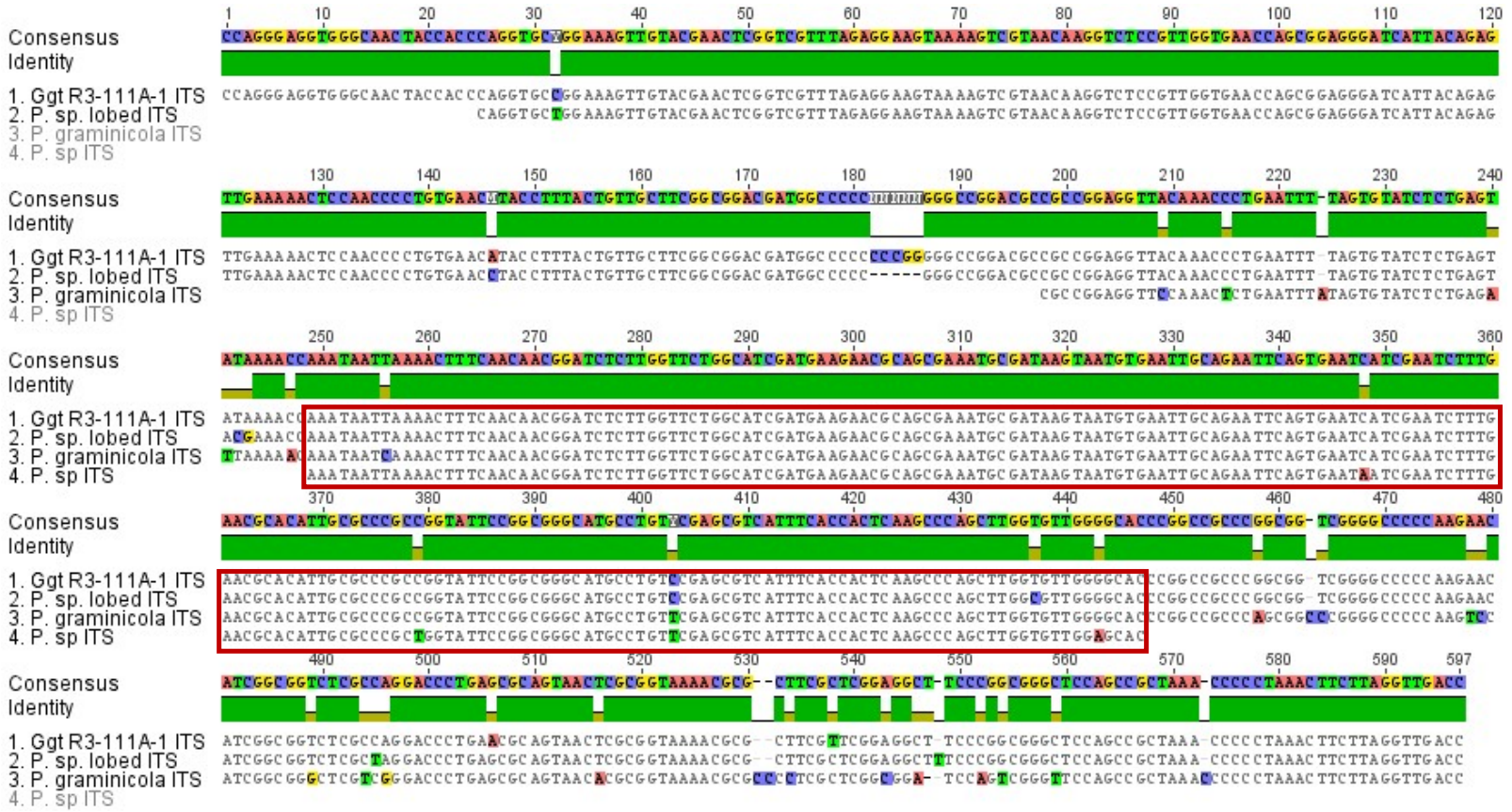


Table 5.9. Assessment of the completeness of the assembled *Phialophora* draft genomes compared to the *Gaeumannomyces graminis* var. *tritici* (*Ggt*) R3-111a-1 annotated genome downloaded from Ensembl Fungi in BUSCO notation (C: complete, [D: duplicated], F: fragmented, M: missing and n: gene number).

| **BUSCO notation assessment results** | **Fungal species** | | | |
|---|---|---|---|---|
| | ***P.* sp. lobed hyphopodia** **Isolate:** N.14.13 | ***Phialophora* sp.** **Isolate:** S.09.13 | ***P. graminicola*** **Isolate:** 74/1736-2 | ***Ggt*** **Isolate:** R3-111a-1 |
| Complete BUSCOs [complete duplicated BUSCOs] | C:28% [D:1.3%] | C:52% [D:2.9%] | C:30% [D:1.8%] | C:97% [D:7%] |
| Fragmented BUSCOs | F:39% | F:34% | F:42% | F:2% |
| Missing BUSCOs | M:31% | M:13% | M:27% | M:0.4% |
| Total BUSCO groups searched | n:1438 | n:1438 | n:1438 | n:1438 |

A Venn diagram was constructed to look at the combinations of complete single-copy BUSCOs, excluding the complete duplicated BUSCOs, of the three *Phialophora* draft genomes and the *Ggt* R3-111a-1 genome (Ensembl Fungi, EMBL-EBI). There were 240 complete BUSCOs in common for all of the three *Phialophora* draft genomes and the *Ggt* genome (Figure 5.7). It is difficult to compare the number of complete single-copy orthologs in common with each individual *Phialophora* genome, as well as a combination of the *Phialophora* species, with the *Ggt* genome since the level of genome completeness varies across the three *Phialophora* draft genomes (Table 5.9 & Figure 5.7). However, the BUSCO assessment revealed a similar level of genome completeness for the *Phialophora* sp. lobed hyphopodia and the *P. graminicola* draft genomes and these two *Phialophora* species also have similar number of complete single-copy orthologs in combination with the *Ggt* BUSCO assessment.

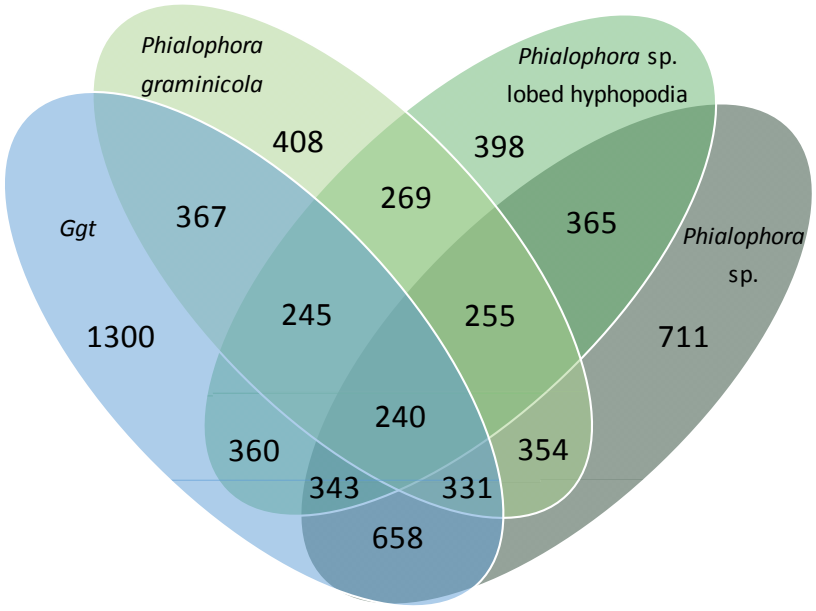



Figure 5.7. Number of complete single-copy BUSCOs of the 1,438 core fungal genes, excluding the complete duplicated BUSCOs, that were found in each of the individual *Phialophora* draft genomes or the *Gaeumannomyces graminis* var. *tritici* (*Ggt*) R3-111a-1 (annotated genome downloaded from Ensembl), that are found in either two species or three species and in common to all four fungal species.

A Venn diagram was then constructed to look at the combinations of complete single-copy BUSCOs, excluding the complete duplicated BUSCOs, that were unique to the individual *Phialophora* draft genomes and the *Ggt* R3-111a-1 genome (Ensembl Fungi, EMBL-EBI) BUSCOs (Figure 5.8). There were difficulties in accurately identifying unique genes due to the differences in the genome coverage and subsequent genome completeness across all four fungal species. The BUSCO assessment revealed that the *Ggt* R3-111a-1 genome had 138 core genes that were missing from the three *Phialophora* draft genomes. Interestingly there were no complete BUSCOs that were only found in either *Phialophora* sp. lobed hyphopodia or *Phialophora* sp., however there was one complete BUSCO found in *P. graminicola* that was missing from the other three genomes (Figure 5.8). The OrthoDB7 2013 database (http://cegg.unige.ch/orthodb7) was used to identify the complete BUSCO in *P. graminicola* (BUSCOfEOG7NGQQW) as a "multiprotein" that is present in 162 of the 175 fungi in the OrthoDB7 2013 database, the gene ontologies are presented in Table 5.10. The *Phialophora* sp. draft genome and the *Ggt* R3-111a-1 genome appeared to have the most core fungal genes in common, although the BUSCO assessment suggested that the *Phialophora* sp. genome was more complete than the *Phialophora* sp. lobed hyphopodia and *P. graminicola* genomes. There were a few core genes that were shown to be common between only *Phialophora* sp. lobed hyphopodia and *Ggt* as well as only between *P. graminicola* and *Ggt*. There were similar numbers of complete single-copy BUSCOs between the combinations of *Ggt* and two of the *Phialophora* species.

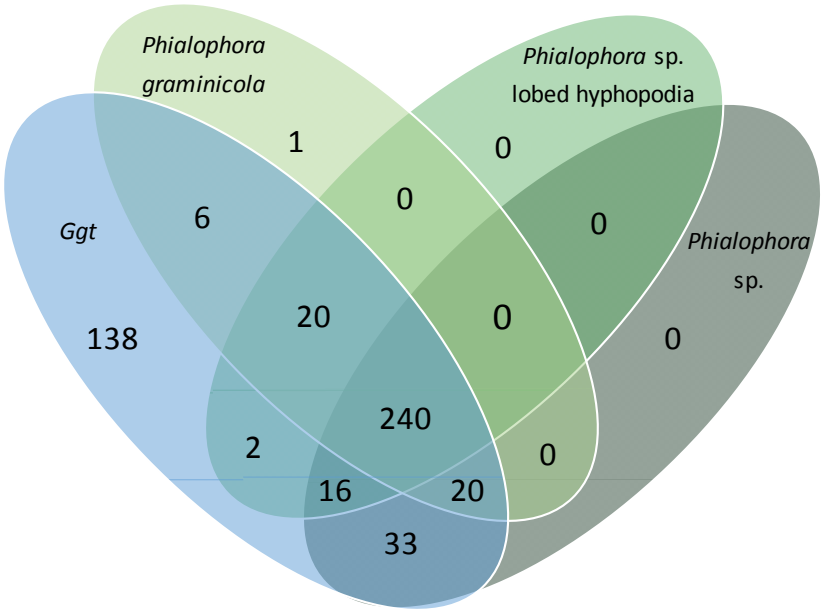



**Figure 5.8.** Unique number of complete single-copy BUSCOs of the 1,438 core fungal genes, excluding the complete duplicated BUSCOs, that were found in each of the individual *Phialophora* draft genomes or the *Gaeumannomyces graminis* var. *tritici* (*Ggt*) R3-111a-1 (annotated genome downloaded from Ensembl), that were specific to either two species or three species and that were common to all four fungal species.

Table 5.10. Gene ontologies of the "multiprotein" identified as a complete BUSCO in the draft *Phialophora graminicola* genome.

| **Gene ontologies** | |
|---|---|
| Molecular function | 161 genes GO:0043565, sequence-specific DNA binding |
| | 18 genes GO:0003713, transcription coactivator activity |
| | 1 gene GO:0003677, DNA binding |
| Biological function | 18 genes GO:0045944, positive regulation of transcription from RNA polymerase II promoter |
| | 14 genes GO:0006351, transcription, DNA-dependent |
| | 8 genes GO:0006355, regulation of transcription, DNA-dependent |
| Cellular component | 17 genes GO:0005739, mitochondrion |
| | 1 gene GO:0005634, nucleus |
| | 1 gene GO:0005730, nucleolus |
| | 1 gene GO:0005829, cytosol |

#### 5.3.2.2 Predicting the gene coding regions of each of the individual *Phialophora* draft genomes

As anticipated, the BUSCO assessment of the genomes did not pick out candidate genes for each of the individual *Phialophora* draft genomes. A BLAST protein analysis for each fungal species against the three remaining species was carried out to identify unique candidate genes for each of the three *Phialophora* species. The number of protein hits for each of the individual *Phialophora* spp. are summarised in Table 5.11. Although *P. graminicola* had the lowest number of protein hits, the blast analysis revealed that the genome had the highest number of absent protein hits across the three *Phialophora* draft genomes. *Phialophora graminicola* had the lowest number of protein hits with either 80-99% identical sites to the two *Phialophora* species and *Ggt* or 100% identical sites to the three fungal species. *Phialophora* sp. lobed hyphopodia and *Phialophora* sp. had a similar number of absent protein hits across all four genomes. These data indicate that there are considerable sequence differences in the predicted protein sets for each species.

Table 5.11. Number of predicted protein hits from the BLAST gene calls of each of the *Phialophora* draft genomes when blasted against the remaining *Phialophora* spp. and *Gaeumannomyces graminis* var. *tritici*.

| **Protein hits** | ***Phialophora* sp. lobed hyphopodia** | ***Phialophora* sp.** | ***Phialophora graminicola*** |
|---|---|---|---|
| **Total no. of protein hits across the 4 genomes** | 12,695 | 13,818 | 11,543 |
| No. of protein hits with >0-19% identical sites | 0 | 0 | 0 |
| No. of protein hits with 20-39% identical sites | 400 | 717 | 829 |
| No. of protein hits with 40-59% identical sites | 1203 | 1687 | 2376 |
| No. of protein hits with 60-79% identical sites | 2718 | 3515 | 3873 |
| No. of protein hits with 80-99% identical sites | 7874 | 7697 | 4371 |
| No. of protein hits with 100% identical sites | 500 | 202 | 94 |
| **Total no. of absent protein hits across the 4 genomes** | 4,628 | 4,836 | 6,067 |

## 5.4 Discussion

### 5.4.1 *Phialophora* isolate collection

This study reports on the isolation and characterisation of a *Phialophora* isolate collection, gathered from three fields across the Rothamsted Farm. A total of nine *Phialophora* cultures were isolated from wheat, eight *Phialophora* sp. lobed hyphopodia isolates and one unnamed *Phialophora* sp. that has previously been isolated at RRes (Ward and Bateman, 1999) and in Germany (Ulrich et al., 2000). A third *Phialophora* species, *P. graminicola* (teleomorph *Gaeumannomyces cylindrosporus*) (Deacon, 1974), was sub-cultured from a cold room stock of isolate 74/1736-2 (Ward and Gray, 1992). The final *Phialophora* isolate collection consisted of three species across ten isolates. Although visual identification of the root colonisation could distinguish the large and single sub-epidermal vesicles of *Phialophora* sp. lobed hyphopodia from the smaller, clustered sub-epidermal vesicles of both *P. graminicola* and the unnamed *Phialophora* sp., difficulties arose when trying to distinguish between the two species with the smaller sub-epidermal vesicles. A reliable molecular assay would therefore be highly beneficial to determine the species identification of *Phialophora* isolates. The contaminant bacterial species was identified in order to allow future isolations to target a more effective antibiotic as well as for the potential of exploring whether the bacterial species is working synergistically with the *Phialophora* fungi.

*Phialophora graminicola* is documented as the most common *Phialophora* species in UK soil (Hornby *et al*. 1998), yet a field isolate was not obtained during this study. *Phialophora graminicola* has previously been isolated from the Rothamsted Farm and cold room stocks at RRes provided a *P. graminicola* isolate that was originally isolated by Richard Gutteridge in 1974 (Ward and Gray, 1992). A pathogenicity assay established that the cold room isolate was still able to colonise wheat. It is unclear why a *P. graminicola* field isolate could not be obtained. The root sterilisation, in sodium hypochlorite, was reduced from 10 minutes to 5 minutes to account for fungal colonisation of the very outer layers of the epidermis, yet still no *P. graminicola* isolate was attained. One explanation could be that the natural *Phialophora* populations of the field have shifted over the past few years and

*Phialophora* sp. lobed hyphopodia could now be the more abundant species. However, *P. graminicola* has been found to be uncommon in cereal crops, unless following grass leys (Deacon, 1973b), and it could therefore be the absence of grass leys in more recent crop rotations on the Rothamsted Farm that has reduced the abundance of *P. graminicola* in the soil.

### 5.4.2 *Phialophora* draft genomes

The *P. graminicola* genome revealed the smallest average insert size across the three species, suggesting that it may have been more difficult to clone. Oddly, this led to the genome containing the highest mean contig size as well as the largest contig size, which contained only one predicted gene. The genome coverage was also significantly higher than the two other *Phialophora* draft genomes, suggesting that the genome assembled well. The *Phialophora* sp. lobed hyphopodia and the unnamed *Phialophora* sp. had similar genome coverages, yet differences in the genome assembly were evident. The *Phialophora* sp. draft genome had the lowest mean contig size as well as the lowest largest contig size, yet this contained the highest number of predicted genes. The genomic G + C content was similar across the three individual *Phialophora* spp. (55-56%) and comparable to the G + C content of *Ggt* (56.85%) and *M. poae* (56.99%), whereas the *M. oryzae* G + C content is slightly lower at 51.61 (Okagaki et al., 2015). The G + C content for the three *Phialophora* draft genomes is considered to be high since the average G + C content for fungal genomes ranges from 32.52-56.97 % (Jung et al., 2008). Li and Du (2014) state that it is currently unclear the relationship between a high G + C content and the genome or chromosome size.

Although the ITS5-ITS4 rDNA extracted sequences from the three *Phialophora* sp. draft genomes was fairly short, the region highlighted the similarity between the three species and the take-all fungus and the need to identify areas of uniqueness within the newly sequenced *Phialophora* genomes. Genome comparisons revealed poor BUSCO completeness values across the three *Phialophora* draft genomes (28-52%) compared to the *Ggt* genome (97%), highlighting the difficulties of completeness with draft genomes. The BUSCO assessment revealed that the *Phialophora* sp. genome had the highest proportion of complete single-copy

orthologs across the three draft genomes. This was unexpected since the *P. graminicola* genome had the greatest number of reads and the greatest genome coverage of the three species, yet a low number of complete single-copy orthologs. However, it is important to note that Simao et al. (2015) stress that there are limitations of gene predictions within the BUSCO assessment and this could potentially inflate the proportions of both fragmented and missing BUSCOs.

There were a large number of proteins absent from the BLAST protein calls of each of the three *Phialophora* draft genomes, between one third to a half of all predicted proteins. The majority of the 'no hits' are likely to have not been assembled rather than being considered truly absent and therefore it is currently too difficult to accurately target the unique regions within the individual *Phialophora* species. It may be better to use the protein hits with a low level of identical sites, such as the 20-39% of identical sites, and then target the regions of polymorphisms within the protein to design species-specific primers in the future. It would be important to target a protein on a large contig and in regions with good read coverage to ensure that the predicted within gene polymorphisms between species are likely to be accurate.

### 5.4.3 Further work

Further genome analysis is in progress to calculate additional information such as the number of predicted tRNA genes and rRNA genes. However, the lack of completeness across the three *Phialophora* draft genomes, highlights the fact that more sequencing is required to obtain a greater number of reads before regions of uniqueness can be accurately identified. More recent sequencing technologies such as Pacific BioSciences (PacBio) Sequencing could be used to overcome some of the limitations of the Illumina HiSeq 2500 sequencing platform (Rhoads and Au, 2015). For example, PacBio sequencing can obtain larger reads which can then provide larger scaffolds in *de novo* genome assembly (Rhoads and Au, 2015). Rob King, RRes bioinformatician, is currently reassembling the *Ggt* R3-111a-1 genome with additional PacBio sequencing. Once this is complete, the newly assembled *Ggt* R3-111a-1 genome will be used to reanalyse the BUSCO assessment and BLAST gene call analysis for the four fungal genomes.

### 5.4.4. Summary

An isolate collection was gathered from fields across the RRes Farm in 2013, obtaining *Phialophora* sp. lobed hyphopodia isolates and an unnamed *Phialophora* sp. isolate. A *P. graminicola* field isolate was not obtained and an isolate was subsequently subcultured from cold room stocks at RRes. Representative isolates for the three *Phialophora* species were sequenced via the Illumina HiSeq 2500 sequencing platform and this chapter reports on the first draft genomes of *Phialophora* species within the *Gaeumannomyces-Phialophora* complex. There were major genome differences across the three *Phialophora* spp., with *P. graminicola* having the best genome coverage. Preliminary analysis has only just begun on the draft genomes but already suggests considerable polymorphism may exist between homologous genes found in all three species. Further genomic analysis will allow the identification and targeting of unique regions within the three *Phialophora* genomes from other species within the *Gaeumannomyces-Phialophora* complex in order to allow the development of a potential molecular diagnostic assay.

# Chapter 6. Evaluation of the ability of *Phialophora* spp. to colonise the roots of cereal genotypes

## 6.1 Introduction

Traditional identification methods for *Phialophora* species in the *Gaeumannomyces-Phialophora* complex rely upon visual assessment of the roots for the characteristic symptoms of runner hyphae and sub-epidermal vesicles. Soil cores are baited with seed in a pot bioassay method (see Chapter 5 for details) and after growth for 5 weeks, the seedling roots are submerged in water in a white dish to assess roots for visual symptoms (Hornby et al., 1998). A number of recording methods can be used. Previous studies at Rothamsted devised a method where seedling seminal roots were cut into 1.5 cm long segments, taken at 2 cm below the seed. The segments were then sterilised in hot 10% sodium hydroxide, acidified with 50% hydrochloric acid and then mounted in lactophenol and examined under a light microscope (Hornby et al., 1998). A 0-3 scale was then used to assess for the presence of sub-epidermal vesicles, where 1 indicates that sub-epidermal vesicles are present in up to 1% of the root tissue but are difficult to visualise under the dissecting microscope, a score of 2 indicates that 1-10% of the root area is occupied by the vesicles and 3 indicates that the vesicles are abundant at above 10% of the root area (Hornby et al., 1998). Gutteridge et al. (2007) used a 0-5 scale to assess wheat seedling roots, from a soil core bioassay, for colonisation under a binocular microscope. A score of 0 indicated no colonisation, 1 indicated the presence of a few sub-epidermal vesicles, 2 indicated most roots colonised with around 1% of the total root area, 3 indicated all roots were colonised with around 2% of the total root area, 4 indicated up to 5% of total root area colonised and 5 up to 10% total root area colonised. A mean score per plant was then calculated for each of the soil cores. Adult plants (GS 69-73) were also assessed for *Phialophora* colonisation. Gutteridge et al. (2007) used two methods, the first assessed the first wheat plant samples on the 0-5 scale as detailed above. The second method followed the method described above for the seedling seminal roots assessment carried out at Rothamsted with the addition of staining the roots with 0.05% trypan blue before

visualising under the light microscope. These traditional identification methods are time consuming and labour intensive. It was decided that the traditional method for the assessment of take-all from pot bioassays (see Chapter 4 for details) and adult plant samples (see Chapter 2) would be used throughout this chapter to assess for *Phialophora* colonisation. These methods would consist of visually assessing, by eye and occasionally with the aid of a microscope, the seedling or adult root systems for the characteristic sub-epidermal vesicles found with *Phialophora* colonisation. This would allow a greater number of samples to be processed in a shorter amount of time without the need of processing the root and staining the roots for visualisation under the microscope.

The two main *Phialophora* species in the *Gaeumannomyces-Phialophora* complex found in agricultural soils are *Phialophora* sp. lobed hypopodia and *Phialophora graminicola*. Both fungi are able to colonise a range of grasses. *Phialophora* sp. lobed hyphopodia (teleomorph: *Gaeumannomyces graminis* var. *graminis*) is pathogenic on rice and Bermuda grass but weakly/non-pathogenic on cereals including wheat (Freeman and Ward, 2004). *Phialophora graminicola* (teleomorph: *Gaeumannomyces cylindrosporus*) is not known to be pathogenic on any of the major cereal species but common hosts in British fields include the grasses Perennial ryegrass (*Lolium perenne*), Italian ryegrass (*Lolium multiflorum*), Cock's-foot (*Dactylis glomerata*), Timothy grass (*Phleum pratense*) and Tall fescue (*Festuca arundinacea*) (Deacon, 1976). The fungus has been found to be uncommon in cereal crops unless following grass leys (Deacon, 1973b). An unnamed *Phialophora* species has also been previously isolated in the UK at Rothamsted Research (Ward and Bateman, 1999) as well from a study in Germany (Ulrich et al., 2000). *Phialophora* spp. compromised an experimental take-all field trial in Fosters field on the Rothamsted Farm in 2005 (McMillan et al., 2011) and more recently have also been found in the Rothamsted fields Pastures, New Zealand, Summerdells 1 and Great Knott 3 (Vanessa McMillan, personal communication). These fields have continued to have detectable levels of *Phialophora* in the soil without grass leys being included in the crop rotation of the field since pre-1987, with the exception of a grass ley included in the crop rotation of the field Pastures in 2008. It is unknown why these

patchy *Phialophora* populations persist with a cereal crop rotation and whether these populations are stable.

The majority of field trials conducted within the *Phialophora* literature focus upon the biocontrol aspect of the species against take-all disease. Field trials conducted in the UK and Australia have examined the effect of the pre-addition of *Phialophora* inoculum to the soil to protect crops against take-all, with little success (Wong and Southwell, 1980, Martyniuk and Myskow, 1984, Gutteridge et al., 2007). Gutteridge et al. (2007) found that natural populations of *Gaeumannomyces cylindrosporus* (*P. graminicola*) were more effective at suppressing take-all disease compared to artificial inoculation of soil. The introduced *Phialophora* spp. in UK arable soils may have had difficulties in establishing viable populations due to the complex biology of the soil and through competition with the natural microbiota (Hornby et al., 1998). Artificial inoculation of *Phialophora* spp. into UK arable fields is not currently feasible. The success of a biocontrol agent relies upon the rapid colonisation of wheat roots by the *Phialophora* spp. and persistence of the fungal species at a high inoculum level during and between cereal crop rotations (Wong, 1975). Take-all inoculum in the soil would need to be at a low level during the *Phialophora* introduction phase, along with a high population of the *Phialophora* biocontrol agent, for successful protection (Gutteridge et al., 2007, Deacon, 1976).

There have been no studies to date which have screened wheat varieties or cereal genotypes to the colonisation ability of natural populations of *Phialophora* in the field, including current elite wheat varieties grown by farmers. If there are differences in the susceptibility or colonisation ability of these elite wheat varieties to *Phialophora* species and their ability to build-up natural populations of *Phialophora* in the soil, the varieties have the potential to form an additional management strategy to help combat take-all disease in second wheats and to understand the *Phialophora* - wheat interaction in more detail.

One of the main objectives in my PhD project was to screen the AHDB Recommended List (RL) of winter wheat varieties for their colonisation by *Phialophora* fungi. A field trial was initially planned for the field season 2013-2014.

Post-harvest soil cores were taken from the reserved field trial site to assess for the presence of *Phialophora* populations. *Phialophora* spp. had previously been identified in the selected field Fosters in 2005 (Vanessa McMillan, personal communication). Unfortunately, the reserved field trial site in Fosters did not contain sufficient levels of *Phialophora* inoculum and the field trial did not go ahead. A *Phialophora* isolate collection was gathered (as described in Chapter 5) and sufficient levels of *Phialophora* species were detected in the field New Zealand. A site was subsequently reserved in the field New Zealand to allow two years of field trials to be conducted on the site. The AHDB 2013/2014 RL was screened in both field seasons, along with the AHDB 2014/2015 RL varieties Zulu and Evolution. McMillan et al. (2011) previously demonstrated that there are differences in the ability of wheat varieties to build-up take-all inoculum in the soil when grown as a first wheat crop and named this the take-all build-up (TAB) trait. This PhD study reports the first experimental data on the ability of elite wheat varieties to build-up *Phialophora* species in the field. Post-harvest soil cores were taken and baited to measure the amount of active *Phialophora* inoculum in the soil that was able to colonise cereal roots after a first wheat crop. The facultative spring wheat variety Cadenza was included and has previously been found as a low builder of take-all inoculum (McMillan et al. 2011), while Hereward is high building. Cadenza and Hereward were included in order to compare the ability of the variety to build-up take-all inoculum with the varieties ability to build-up *Phialophora* inoculum. This chapter evaluates the 2014-2015 field trial season results.

As well as evaluating the susceptibility of wheat varieties to *Phialophora* fungi at the adult plant stage, a working pot bioassay was developed in order to screen cereal genotypes for *Phialophora* susceptibility at the seedling stage. *Phialophora* isolates were used from the isolate collection, gathered in Chapter 5, to screen the cereal genotypes for *Phialophora* colonisation. The pot bioassay method was devised to screen cereals at a 50% level of *Phialophora* colonisation. The aim to achieve ~50% roots infected was based on the take-all pot bioassay protocol. A 50% level of colonisation should allow good discrimination between varieties in their susceptibility to *Phialophora* colonisation in experimental pot bioassays. Cereal

genotypes were assessed in seedling pot bioassays for their susceptibility to *Phialophora* species as part of an AHDB summer bursary project with student Brittany Burton. I devised a 10-week bursary project to evaluate a number of cereal genotypes used in the take-all field trials: Watkins line 777 and Paragon (Chapter 3) and the *Triticum monococcum* mapping population parental lines MDR037 and MDR046 (Chapter 4) for susceptibility to *Phialophora* fungi. The standard take-all control genotypes were also included, oats (resistant to *Ggt*), rye (highly resistant to *Ggt*), triticale (moderately resistant to *Ggt*) and the hexaploid wheat variety Hereward (highly susceptible to *Ggt*). A pot bioassay method was devised to screen whether cereal genotypes that are partially resistant or susceptible to take-all infection are more or less susceptible to *Phialophora* colonisation.

## 6.2 Materials and methods

### 6.2.1 Susceptibility of elite wheat to *Phialophora* spp. field trials

Two field trials to evaluate the susceptibility of elite wheat varieties to *Phialophora* spp. were set up during the field seasons of 2014-2015 and 2015-2016 in the field New Zealand on the Rothamsted Farm (Table 6.1). The two trials were drilled as 1$^{st}$ wheats after a previous break crop of winter oilseed rape. The winter oilseed rape drilled for the reserved site for the 2015-2016 field trial (2016/R/WW/1620) had poor establishment (Figure 6.1) and was subsequently sprayed off with glyphosphate and ploughed in on 03/06/15 before the site was then drilled with spring oats on the 29/06/15 to establish a uniform crop and to ensure nitrogen levels were consistently low throughout the site. The spring oats were then sprayed off with glyphosphate and flailed just prior to ploughing. The two trials consisted of five replicates of 40 elite wheat varieties in a randomised block design, calculated by statistician Rodger White in GenStat (VSNI, Hemel Hempstead, UK). Field trial plans can be seen in Appendix 6.1. The AHDB 2013/2014 Recommended List (RL) of 37 winter wheat varieties, with the exception of Gravitas (seed was not available), were evaluated in both trials, along with the two additional candidate winter wheat varieties Zulu and Evolution provided by Limagrain (Table 6.2). The spring wheat variety Cadenza has previously been found to be a low builder of take-all inoculum (McMillan et al., 2011) and was included in the two trials to establish the capability

of this variety to build-up *Phialophora* spp. The winter wheat variety Hereward was included as a highly susceptible control variety, this variety has previously been found to be a high builder of take-all inoculum (McMillan et al., 2011).



**Figure 6.1.** Aerial photograph of 2014-2015 (2015/R/WW/1516) field trial (right) and reserved site of winter oilseed rape for trial 2015-2016 (2016/R/WW/1620) field trial (left) in New Zealand field. Poor establishment of the winter oilseed rape crop is visible towards the top of the site (photograph credit: Visual Communications Unit at Rothamsted Research).



**Figure 6.2.** Photograph of 2014-2015 (2015/R/WW/1516) field trial taken on 24/06/15.

Above-ground phenotypic crop measurements of plant height, days to anthesis and days to leaf senescence were recorded for each plot (Table 6.3) (see Chapter 2). The above-ground phenotypic crop measurements were analysed by statistician Rodger White using analysis of variance (ANOVA) in GenStat. Plant samples were taken in July from all plots to determine the susceptibility of the elite wheat varieties to *Phialophora* fungi (Table 6.3) (see Chapter 2). Yields were taken from each plot by the Rothamsted Farm and analysed by statistician Rodger White using a Residual Maximum Likelihood (REML) variance components analysis in GenStat.

Table 6.1. Experimental field trial design used to evaluate the elite wheat varieties to *Phialophora* spp.

| Field trial | Rothamsted Field and soil type | Previous crop/crops | Number of varieties/ replication | Plot size | Seed rate (seeds/m²) | Sowing date | Harvest date |
|---|---|---|---|---|---|---|---|
| 2015/R/WW/1516 | New Zealand Typical Batcombe | Winter oilseed rape | 40 x 5 reps | 6 rows x 1m | 350 | 10/10/2014 | 17/09/2015 |
| 2016/R/WW/1620[1] | New Zealand Typical Batcombe | Winter oilseed rape then spring oats | 40 x 5 reps | 6 rows x 1m | 350 | 02/10/2015 | 09/08/2016 |

[1] Field trial 2016/R/WW/1620 was not conducted within the PhD project but will be used to confirm 2015/R/WW/1516 results.

Table 6.2. Elite wheat varieties used in 2014-2015 (2015/R/WW/1516) and 2015-2016 (2016/R/WW/1620) field trials.

| Variety | Nabim group | Breeder provided seed | Parentage | Year first listed on AHDB RL |
|---|---|---|---|---|
| Alchemy | 4 | Limagrain | Claire x (Consort x Woodstock) | 2006 |
| Beluga | 4 | Senova | (Robigus x Canterbury) x Claire | 2010 |
| Cadenza[1] | 2 | - | Axona x Tonic | 1994 |
| Chilton | 2 | DSV | Solstice x Option | 2013 |
| Claire | 3 | Limagrain | Wasp x Flame | 1999 |
| Cocoon | 3 | Agrii | Wizard x Xi19 | 2011 |
| Conqueror | 4 | KWS | Robigus x Equinox | 2010 |
| Cordiale | 2 | KWS | (Reaper x Cadenza) x Malacca | 2004 |
| Cougar | 4 | RAGT | Tuscan x Robigus | 2013 |
| Crusoe | 1 | Limagrain | Cordiale x Gulliver | 2012 |
| Delphi | 3 | Senova | Gladiator x Robigus | 2013 |
| Denman | 4 | Syngenta | Alchemy x Glasgow | 2011 |
| Dickens | 4 | Agrii | (Equinox x Charger) x Defender | 2013 |
| Duxford | 4 | Syngenta | Solstice x Scorpion 25 | 2008 |
| Einstein | 2 | Limagrain | (NHC49 x UK yield bulk) x (Haven x Clarion) | 2003 |
| Evolution | 4 | Limagrain | Smuggler x (Robigus x SJ5558) | 2014 |
| Gallant | 1 | Syngenta | (Malacca x Charger) x Xi19 | 2009 |
| Grafton | 4 | KWS | Cordiale x CPBT W97 | 2009 |
| Hereward[2] | 1 | - | Norman 'sib' x Disponent | 1991 |
| Horatio | 4 | Limagrain | Alchemy x Oakley | 2012 |
| Invicta | 3 | Limagrain | NSL WW48 x Robigus | 2010 |
| JB Diego | 4 | Senova | 3351b2 x Stru2374 | 2008 |
| KWS Croft | 3 | KWS | (Deben x Robigus) x Robigus | 2013 |

Table 6.2. Continued.

| Variety | Nabim group | Breeder provided seed | Parentage | Year first listed on AHDB RL |
|---|---|---|---|---|
| KWS Gator | 4 | KWS | Oakley x CPBT W89 | 2012 |
| KWS Kielder | 4 | KWS | Brompton x Oakley | 2013 |
| KWS Podium | 2 | KWS | (Xi19 x Robigus) x Cordiale | 2011 |
| KWS Santiago | 4 | KWS | Sherbourne x Oakley | 2011 |
| KWS Sterling | 2 | KWS | Cordiale x (Quest x Wizard) | 2010 |
| KWS Target | 3 | KWS | (CPBT W89 x Robigus) x Robigus | 2011 |
| Leeds | 4 | KWS | Robigus x Istabraq | 2013 |
| Monterey | 3 | Senova | Istabraq x Robigus | 2013 |
| Myriad | 4 | Limagrain | NSL 00-0742 x Nijinski | 2013 |
| Panorama | 2 | Limagrain | Solstice x Xi19 | 2009 |
| Relay | 4 | RAGT | Gladiator x Vector | 2012 |
| Revelation | 4 | Limagrain | (Alchemy x Claire) x Shepherd | 2013 |
| Scout | 3 | Senova | Z435 x Deben | 2009 |
| Solstice | 1 | Limagrain | Vivant x Rialto | 2002 |
| Tuxedo | 3 | RAGT | (Goodwill x Robigus) x Gladiator | 2011 |
| Viscount | 4 | KWS | Robigus x Canterbury | 2009 |
| Zulu | 3 | Limagrain | (CEB99080 X Claire) x Robigus | 2014 |

[1] Cadenza seed provided from previous field trial on the Rothamsted Farm 2014/R/CS/750, plot 42.

[2] Hereward seed provided from previous field trial on the Rothamsted Farm 2013/R/WW/1316.

Table 6.3. Assessment and sampling methodology for 2014-2015 field trial (2015/R/WW/1516).

| Field trial | Sampling measurements | Units per plot | Date sampled | Growth stage (GS) |
|---|---|---|---|---|
| 2015/R/WW/1516 | Ear emergence | GS | 02/06/15 - 19/06/15 | 61 |
| | Leaf senescence | 0-10 | 23/07/15 - 17/08/15 | 83-92 |
| | Plant heights | cm | 24/06/15 | 73-75 |
| | Plant samples | 3 x 20cm | 07/07/15 - 08/07/15 | 75-77 |
| | Post-harvest soil cores | 6 cores | 02/09/2015 - 03/09/15 | - |

Post-harvest soil cores were taken from each plot in order to set up a soil core bioassay to gauge the amount of *Phialophora* fungi that had built up in the soil under the different elite wheat varieties and to establish the ability of the *Phialophora* fungi to colonise seedling wheat roots. Soil cores (5.5 cm diameter by 10 cm deep; 6 cores per plot) were taken in a zig-zag transect from different rows across the plots using an auger. The soil cores were inverted into plastic drinking cups (7.5 cm diameter x 11 cm tall, drilled with 4 drainage holes, 3mms diameter) containing a 50 $cm^3$ layer of damp coarse sand. The soil cores were transported back to the field laboratory and were stored in the cold room at 5°C until processed over the following months. A total of 1200 soil cores were taken and these were separated into three sets (A, B and C) in order to process. Each of the three sets contained two soil cores from each plot. One of the soil cores was baited with the same variety as the field plot. The second soil core was baited with the control variety Hereward in order to determine whether there was a bias for variety. Each of the three sets were set up in a randomised design for plot and treatment prior to placing in the controlled environment room. The soil cores were sprayed with water and ten seeds of each variety were placed on top of the soil. Seeds were covered with ~2 cm layer of horticultural grit and cores were placed in a controlled environment room (16 hour day, 70% relative humidity, 15°C day/10°C night, twice weekly watering) for five weeks. After five weeks the roots were washed free of soil and immersed in a white dish to visually examine the roots for *Phialophora* colonisation. Roots were examined for the presence of sub-epidermal vesicles. The total number of plants and roots and the number of *Phialophora* colonised plants and roots were recorded in order to calculate percentages. Take-all disease symptoms, consisting of black necrotic lesions, were also recorded where present. The *Phialophora* colonisation percentages and the take-all percentages were analysed by statistician Rodger White using a REML variance components analysis. A random sub-set of between 5-10 pots, per set (A, B, C), that exhibited highly colonised roots were examined under the stereomicroscope (LEICA M205 FA) to confirm the presence of sub-epidermal vesicles from the visual assessments.

A subset of 19 pots, across all three sets, that exhibited roots that were highly colonised with *Phialophora* were selected to isolate the *Phialophora* spp. to confirm visual assessments and to identify what *Phialophora* spp. were present in the field site. The isolation method was followed from Chapter 2, with the exception of performing the extractions on fresh mycelium scraped from plates on the same day as the extractions and one 5 mm steel ball bearing was added to the tube once the extraction buffer had been added. Samples were homogenised in a fastprep-24™ (MP Biomedicals, USA) at 5 m/sec for 45 secs before incubating the samples in the waterbath at 65°C for 30 mins (see Chapter 2). PCR was performed to amplify internal transcribed spacer (ITS)5 F (GGAAGTAAAAGTCGTAACAAGG) and ITS4 R (TCCTCCGCTTATTGATATGC) regions and the PCR products were then purified using the Qiagen QIAquick PCR Purification Kit (Qiagen Ltd., Manchester, UK), all detailed in Chapter 2. Sequences were sent for sequencing analysis as detailed in Chapter 2. Returned ITS sequences were then blasted in the NCBI BLAST tool for species identification.

A phylogenetic tree was constructed to identify the genetic relationship between the *Phialophora* spp. field isolates from 2015/R/WW/1516 with the *Phialophora* isolate collection gathered in Chapter 5. The primer ends were trimmed and a 511 base pair (bp) region of the sequenced ITS5-ITS4 rDNA regions of the field isolates of trial 2015/R/WW/1516 and ITS5-ITS4 rDNA regions of the *Phialophora* field isolate collection gathered in Chapter 5 (see Table 5.7) were aligned in the software package Geneious (Biomatters Ltd. v8.1.3) with the 511 bp ITS regions obtained from the NCBI nucleotide database for: the top three BLAST hits of species identification for the 2015/R/WW/1516 field isolates, reference *Phialophora* spp. sequences, reference *Ggt* sequences and *Magnaporthe grisea* as the outlier group. A phylogenetic tree was then constructed on the alignment using the genetic distance model of Tamura-Nei, the tree build method of neighbour-joining with 1000 bootstrap replicates and a support threshold set at 75% in Geneious (Biomatters Ltd. v8.1.3). The Interactive Tree of Life (ITOL) (http://itol.embl.de/) was used to illustrate the phylogenetic analysis.

### 6.2.2 Susceptibility of cereal genotypes to *Phialophora* spp. in seedling pot bioassays

Sand maizemeal inoculum was prepared in order to carry out pot bioassays to screen the susceptibility of nine cereal genotypes to *Phialophora* spp. at the seedling stage. Inoculum was prepared for two *Phialophora* species from the isolate collection, *Phialophora* sp. (isolate S.09.13) and *Phialophora* sp. lobed hyphopodia (isolate N.14.13) (see Chapter 5, Table 5.7). Two 250 ml conical flasks were filled with 100 g silver sand, 3 g maizemeal and 10 ml sterile distilled water for each isolate and a rubber bung was placed into the top of the flask and covered with a layer of tin foil. Flasks were autoclaved twice, with a 48 hr period in between. Three 1 $cm^2$ agar plugs were cut from confluent fungal PDA plates using a sterile tooth pick and added to the flasks. The cultures were left at room temperature for 6 weeks and shaken weekly to aid even-colonisation. Inoculum was stored at 4°C for future use.

Two pot bioassays were prepared to calibrate the *Phialophora* sand maizemeal inoculum for the two isolated *Phialophora* species, *Phialophora* sp. (isolate S.09.13) and *Phialophora* sp. lobed hyphopodia (isolate N.14.13). A pot bioassay was set-up with coarse sand and another using soil to test the best growth medium for colonisation. Soil was previously collected in September 2013 from Great Harpenden I (after oats), crumbled and sieved for use in the pot bioassays within this chapter. A series of dilutions were carried out of sand maizemeal inoculum to silver sand (1:50, 1:100, 1:150, 1:200, 1:250, 1:300 and 1:350) for both *Phialophora* species. A randomised block design was calculated in GenStat (VSNI, Hemel Hempstead, UK) and included five replicates for each of the seven inoculum dilutions and three replicates of an experimental negative control for each of the two pot bioassays.

Plastic drinking cups (7.5 cm diameter x 11 cm tall, drilled with 4 drainage holes, 3mms diameter) were filled with 50 $cm^3$ of damp coarse sand. A mixture of 50 g of each sand maizemeal inoculum: silver sand dilution and 250 g of either soil or coarse sand was shaken in a plastic bag and poured into the cups. The pots were watered and 10 seeds (var. Hereward) were placed on top of the soil surface. A 2

cm² layer of horticultural grit was poured on top and pots were placed in a controlled environment room (16 hour day, 70% relative humidity, 15°C day/10°C night, twice weekly watering) for 5 weeks. After 5 weeks the roots were washed free of soil and immersed in water in a white dish to examine and record the number roots with visible sub-epidermal vesicles as well as assessing the number of roots with visible sub-epidermal vesicles when viewed under a light microscope. The total number of plants and roots and the number of *Phialophora* colonised plants and roots were recorded in order to calculate percentages. The percentages of *Phialophora* colonised roots were transformed using the logit transformation to ensure equal variance. Transformed data was statistically analysed using REML variance components analysis and tables of predicted means were calculated.

In June 2014 a 10 week AHDB summer bursary project, with undergraduate student Brittany Burton, evaluated the susceptibility of cereal genotypes to the selected *Phialophora* sp. lobed hyphopodia isolate and a *Phialophora* sp. isolate used in the calibration above. Cereal genotypes tested were: oats, rye, triticale, Hereward, Paragon, Watkins Collection line 777 and *Triticum monococcum* accessions MDR037 and MDR046. The cereal genotypes oats, rye, triticale and Hereward are traditionally used as control genotypes to benchmark the level of take-all in the field and pot bioassays (McMillan et al., 2014) and were included to compare levels of colonisation found for the *Phialophora* species. Oats are resistant to take-all, rye is highly resistant to take-all, triticale represents moderate resistance to take-all and Hereward is used as our highly susceptible control for take-all infection. The cereal genotypes Paragon and Watkins line 777 were previously screened in Chapter 3 for take-all susceptibility, Paragon was found to be susceptible to take-all and Watkins line 777 partially resistant. The *T. monococcum* accessions MDR037 (S) and MDR046 (R) were the parental lines of the mapping population screened in Chapter 4. These four cereal genotypes were included in the experimental pot bioassays in order to compare their susceptibility to take-all with their susceptibility to *Phialophora* fungi. Hereward was also used as a negative control in both pot bioassays with non-inoculated potato dextrose agar (PDA) or sand maizemeal. Two methods of inoculation were undertaken to evaluate and compare the

effectiveness of each method, a sand maizemeal inoculum pot bioassay and a PDA plate inoculum pot bioassay. A randomised block design was calculated in GenStat (VSNI, Hemel Hempstead, UK) and included three inoculated replicates for each cereal genotype and three replicates of Hereward as an experimental negative control for each of the two *Phialophora* isolates. Plastic drinking cups (7.5 cm diameter x 11 cm tall, drilled with 4 drainage holes, 3 mms diameter) were filled with a 50 $cm^3$ layer of damp coarse sand. The sand maizemeal inoculum pot bioassay method consisted of an inoculum dilution of 1:10 (sand maizemeal inoculum: silver sand) was prepared for *Phialophora* sp. lobed hyphopodia and a 1:25 (sand maizemeal inoculum: silver sand) dilution for the *Phialophora* sp. isolate and 50 g of each inoculum was mixed with 250g of soil and poured into the pots. The negative control pots for the sand maizemeal pot bioassay were prepared by adding 250 g of soil and then 50 g of silver sand. The PDA plate inoculum pot bioassay consisted of macerating $1/6^{th}$ of a confluent PDA plate of either *Phialophora* sp. lobed hyphopodia or *Phialophora* sp. with soil, equating to a 25 g layer. Colonised PDA plates were previously used in Chapter 5 to identify isolated fungi and results found that 1/3rd of a confluent plate produced too high a level of colonisation of the wheat seedling roots and would not allow us to discriminate any differences between the cereal genotypes in their level of susceptibility so this was reduced to 1/6th of a plate for the experimental pot bioassays in this chapter. The negative control pots were prepared by macerating $1/6^{th}$ of a non-colonised PDA plate with soil, equating to a 25 g layer. A further 50 g of soil was added on top. Pots were sprayed with water and ten seeds of each variety were placed on the soil surface. Seeds were covered with ~2 cm layer of horticultural grit and cores were placed in a controlled environment room (16 hour day, 70% relative humidity, 15°C day/10°C night, twice weekly watering) for five weeks. After five weeks the roots were washed free of soil and immersed in a white dish to visually examine the roots for *Phialophora* colonisation by Brittany Burton. Roots were examined for the presence of sub-epidermal vesicles. The total number of plants and roots and the number of *Phialophora* colonised plants and roots were recorded in order to calculate percentages. A repeat pot bioassay using the PDA plate inoculum method was carried out in December 2014. The *Phialophora* colonisation percentages were

transformed using the logit transformation to ensure equal variance for each individual experimental pot bioassay. Transformed data was then statistically analysed using ANOVA. The *Phialophora* colonisation percentages for each of the experimental pot bioassays were then pooled together and a combined statistical analysis using a REML variance components analysis was carried out. Predicted tables of means were calculated.

## 6.3 Results

### 6.3.1 Performance of elite wheat varieties to *Phialophora* spp.in the field

A subset of ten plots, chosen due to high levels of *Phialophora* colonisation found within the assessed soil core bioassays, were visually assessed for *Phialophora* colonisation. No sub-epidermal vesicles could not be seen by eye within the adult root systems (Figure 6.3), or under the light microscope, and the remaining samples were not assessed.



Figure 6.3. Photographs of whole adult plant root system of Duxford on the left (plot 141) and KWS Kielder on the right (plot 24) from 2015/R/WW/1516 field trial. (Photograph credit: Graham Shephard, Photographer at Rothamsted Research).

The soil core bioassays were assessed and a significant difference in the ability of the elite wheat varieties to build-up inoculum of natural *Phialophora* species was found (Table 6.4). *Phialophora* colonisation was found for all elite varieties across the two baiting methods. A significant interaction was found for the baiting method across the varieties, with a trend for a higher percentage of roots colonised when baited with Hereward for the majority of the elite varieties (REML Hereward baited x field plot variety baited interaction: $P$ = <0.001) (Table 6.4). Post-hoc analysis revealed that the 40 elite wheat varieties could be divided into three distinct groups. The first grouping of varieties can be seen to have a very low percentage of *Phialophora* colonisation when baited back on to the field plot variety but a higher percentage of *Phialophora* colonisation when baited on to Hereward (indicated in blue), for example, Alchemy, Evolution, Zulu and Leeds (Figure 6.4). The second group of varieties are seen to have a similar percentage of *Phialophora* colonisation regardless of baiting variety (indicated in green), for example Cadenza, Duxford and the control Hereward (Figure 6.4). The third group had a higher percentage of *Phialophora* colonisation when baited back on to the field plot variety (indicated in red), for example JB Diego and Scout (Figure 6.4). There was no correlation found between the percentage of roots colonised with *Phialophora* when baited with Hereward and the percentage of roots colonised with *Phialophora* when baited with the field plot variety ($r_s$ = 0.119, $P$ = 0.116, n = 40) (Figure 6.4).

Table 6.4. *Phialophora* colonisation of seedling roots grown in soil cores baited with Hereward or the field plot variety after the harvest of the elite wheat varieties of the first wheat field trial 2015/R/WW/1516 and ranking of the varieties on the 2013/2014 AHDB RL of old to new.

| **Variety** | **Logit percentage of roots colonised with *Phialophora* baited with Hereward (back transformed means)** | | **Logit percentage of roots colonised with *Phialophora* baited with the field plot variety (back transformed means)** | | **Year first listed on AHDB RL** | **Ranking order of old to new varieties on 2013/2014 RL** |
|---|---|---|---|---|---|---|
| Alchemy | -1.755 | (2.40) | -2.260 | (0.58) | 2006 | 7 |
| Beluga | -1.575 | (3.61) | -2.197 | (0.72) | 2010 | 10 |
| Cadenza | -1.318 | (6.18) | -1.356 | (5.73) | 1994 | 2 |
| Chilton | -1.469 | (4.53) | -2.360 | (0.38) | 2013 | 13 |
| Claire | -1.153 | (8.55) | -2.453 | (0.24) | 1999 | 3 |
| Cocoon | -0.824 | (15.64) | -2.167 | (0.79) | 2011 | 11 |
| Conqueror | -1.276 | (6.74) | -2.120 | (0.92) | 2010 | 10 |
| Cordiale | -1.370 | (5.57) | -1.040 | (10.61) | 2004 | 6 |
| Cougar | -1.324 | (6.11) | -2.269 | (0.56) | 2013 | 13 |
| Crusoe | -1.251 | (7.07) | -2.094 | (1.00) | 2012 | 12 |
| Delphi | -1.391 | (5.33) | -2.382 | (0.35) | 2013 | 13 |
| Denman | -1.425 | (4.97) | -2.074 | (1.06) | 2011 | 11 |
| Dickens | -1.365 | (5.63) | -1.780 | (2.26) | 2013 | 13 |
| Duxford | -1.156 | (8.52) | -1.253 | (7.04) | 2008 | 8 |
| Einstein | -1.382 | (5.43) | -1.056 | (10.28) | 2003 | 5 |
| Evolution | -1.106 | (9.37) | -1.702 | (2.72) | 2014 | 14 |
| Gallant | -1.407 | (5.16) | -1.419 | (5.03) | 2009 | 9 |
| Grafton | -1.299 | (6.43) | -1.771 | (2.31) | 2009 | 9 |
| Hereward | -1.109 | (9.31) | -1.134 | (8.88) | 1991 | 1 |
| Horatio | -1.110 | (9.21) | -2.349 | (0.40) | 2012 | 12 |
| Invicta | -1.169 | (8.30) | -2.277 | (0.54) | 2010 | 10 |
| JB Diego | -1.289 | (6.55) | -0.905 | (13.56) | 2008 | 8 |
| KWS Croft | -0.851 | (14.92) | -2.067 | (1.08) | 2013 | 13 |
| KWS Gator | -1.188 | (8.01) | -1.333 | (6.00) | 2012 | 12 |
| KWS Kielder | -0.923 | (13.15) | -1.043 | (10.55) | 2013 | 13 |
| KWS Podium | -1.238 | (7.25) | -1.520 | (4.07) | 2011 | 11 |
| KWS Santiago | -1.248 | (7.11) | -2.082 | (1.03) | 2011 | 11 |
| KWS Sterling | -0.887 | (13.99) | -2.191 | (0.73) | 2010 | 10 |
| KWS Target | -1.062 | (10.17) | -1.834 | (1.99) | 2011 | 11 |
| Leeds | -0.701 | (19.25) | -2.116 | (0.93) | 2013 | 13 |
| Monterey | -0.906 | (13.54) | -1.902 | (1.68) | 2013 | 13 |
| Myriad | -1.125 | (9.04) | -2.117 | (0.93) | 2013 | 13 |
| Panorama | -1.086 | (9.72) | -1.333 | (5.99) | 2009 | 9 |
| Relay | -1.202 | (7.78) | -1.089 | (9.67) | 2012 | 12 |

Table 6.4. Continued.

| Variety | Logit percentage of roots colonised with Phialophora baited with Hereward (back transformed means) | | Logit percentage of roots colonised with Phialophora baited with the field plot variety (back transformed means) | | Year first listed on AHDB RL | Ranking order of old to new varieties on 2013/2014 RL |
|---|---|---|---|---|---|---|
| Revelation | -0.949 | (12.53) | -2.017 | (1.24) | 2013 | 13 |
| Scout | -1.032 | (10.76) | -0.859 | (14.71) | 2009 | 9 |
| Solstice | -1.195 | (7.89) | -1.021 | (10.99) | 2002 | 4 |
| Tuxedo | -0.996 | (11.51) | -1.721 | (2.60) | 2011 | 11 |
| Viscount | -1.340 | (5.92) | -2.300 | (0.50) | 2009 | 9 |
| Zulu | -0.828 | (15.53) | -1.802 | (2.15) | 2014 | 14 |
| d.f. | 39 | | 39 | | | |
| SED | 0.2311 | | 0.2311 | | | |
| F statistic | 5.58 | | 5.58 | | | |
| F probability | <0.001 | | <0.001 | | | |
| Grand mean | -1.182 | (8.09) | -1.769 | (2.32) | | |

The percentage data was then correlated against the nabim groups of the RL varieties. No correlation was found between the nabim groups and the percentage of roots colonised with *Phialophora* when baited with the variety Hereward ($r_s$ = -0.119, *P* = 0.056, n = 40) or the percentage of roots colonised with *Phialophora* when baited with the field plot variety ($r_s$ = 0.029, *P* = 0.204, n = 40). The percentage data was also correlated against the year that the variety was first listed on the AHDB RL, the year data was ranked from the oldest (1) to the newest (14) varieties (Table 6.4). A weakly negative correlation was found between the ranked year data that the variety was first listed on RL and the percentage of roots colonised with *Phialophora* when baited with the plot variety ($r_s$ = -0.278, *P* = 0.017, n = 40) (Figure 6.5). Yet a moderately positive correlation was found between the ranked year data that the variety was first listed on RL and the percentage of roots colonised with *Phialophora* when baited with the variety Hereward ($r_s$ = 0.328, *P* = 0.011, n = 40) (Figure 6.6).

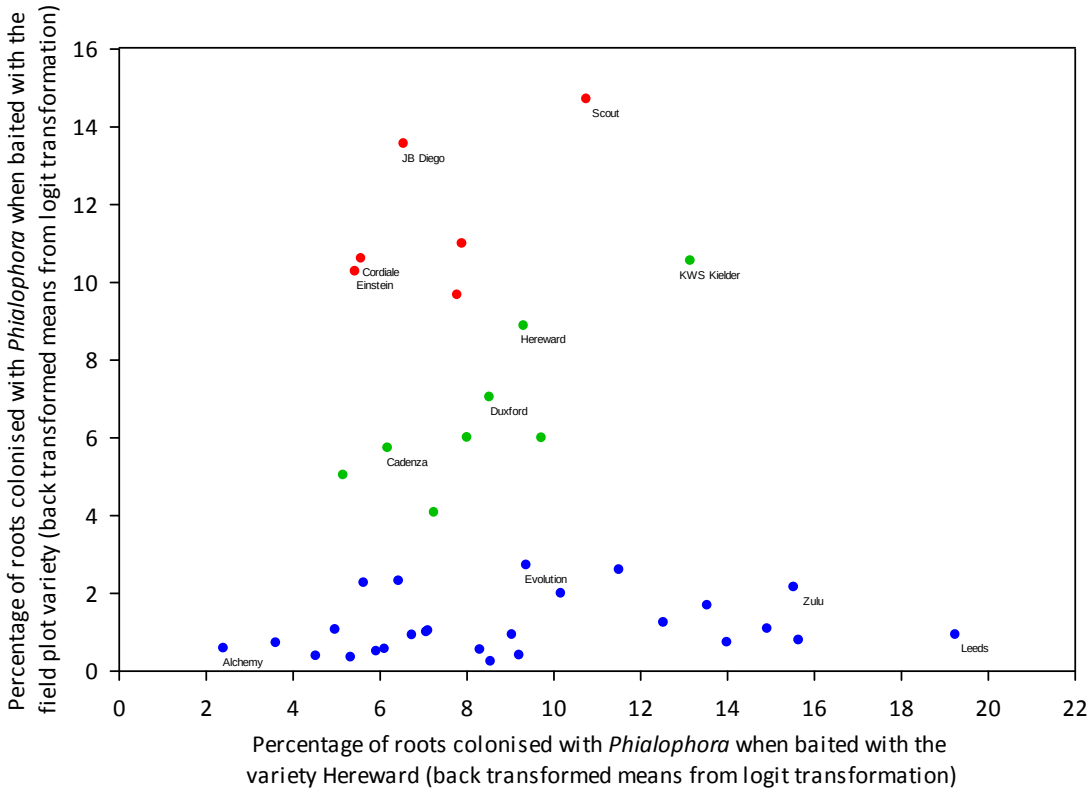



**Figure 6.4.** Correlation between the percentage of roots colonised with *Phialophora* when baited with either Hereward or the field plot variety after the harvest of the elite wheat varieties of the first wheat field trial 2015/R/WW/1516. $r_s = 0.119$, $P = 0.116$, n = 40. The elite wheat varieties were visually divided into three groups, varieties with a higher percentage of colonised roots when baited with Hereward (illustrated in blue), varieties with similar levels of colonisation with either baiting methods (illustrated in green) and varieties with a higher level of colonisation when baited with the field variety (illustrated in red).

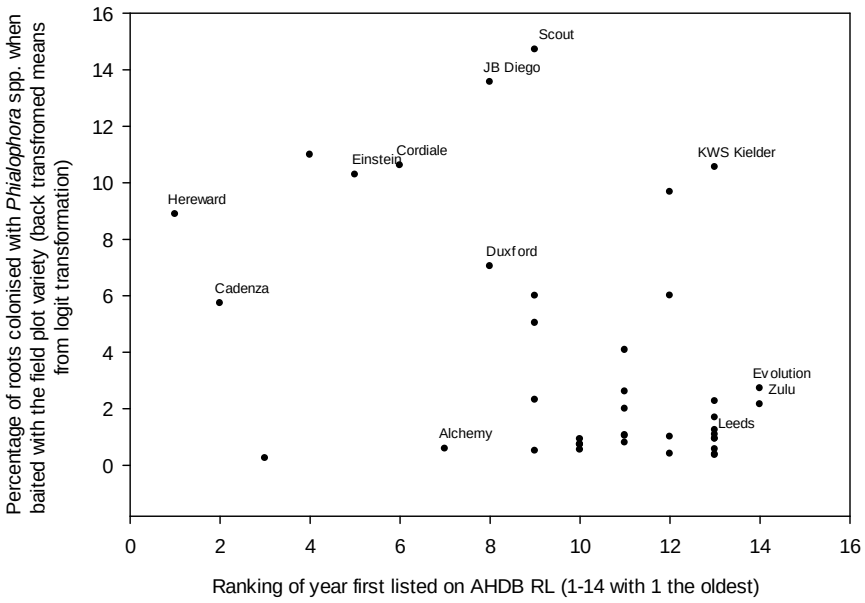



Figure 6.5. Correlation between the ranking of the year that the variety was first listed on the AHDB RL and the percentage of roots colonised with *Phialophora* when baited with the field plot variety. $r_s$ = -0.278, *P* = 0.017, n = 40.

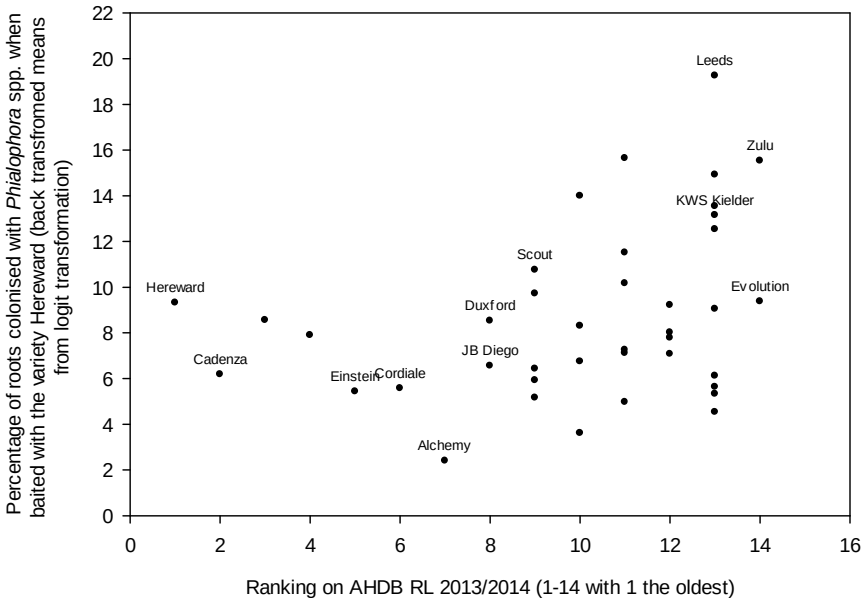



Figure 6.6. Correlation between the ranking of the year that the variety was first listed on the AHDB RL and the percentage of roots colonised with *Phialophora* when baited with the variety Hereward. $r_s$ = 0.328, *P* = 0.011, n = 40.

The soil core pot bioassay seedlings were also assessed for take-all infection. As expected, there was an extremely low level (grand mean = 0.39%) of take-all infection across all varieties (Table 6.5). There was a significant difference in the percentage of roots infected with take-all across all varieties, regardless of baiting method (REML: *P* = <0.001) (Table 6.5). The varieties Alchemy and Relay had the highest percentage of roots infected with take-all, whereas Delphi and Leeds had the lowest level of roots infected with take-all and Grafton had no take-all infection across either baiting method. There was no significant difference of the percentage of roots infected with take-all when baited onto Hereward or the field plot variety (REML Hereward baited x field plot variety baited interaction: *P* = 0.539, SED = 0.236, d.f. = 39). There was no correlation found between the percentage of roots colonised with *Phialophora* when baited onto Hereward and the percentage of roots infected with take-all ($r_s$ = 0.064, *P* = 0.175, n = 40) or between the percentage of roots colonised with *Phialophora* when baited onto the field plot variety and the percentage of roots infected with take-all ($r_s$ = 0.206, *P* = 0.051, n = 40).

Table 6.5. Take-all infection of soil cores baited with the variety Hereward or the field plot variety after the harvest of the elite wheat varieties of the first wheat field trial 2015/R/WW/1516.

| Variety | Logit percentage of roots infected with take-all (back transformed means) | |
|---|---|---|
| Alchemy | -2.048 | (1.14) |
| Beluga | -2.579 | (0.07) |
| Cadenza | -2.107 | (0.96) |
| Chilton | -2.514 | (0.15) |
| Claire | -2.369 | (0.37) |
| Cocoon | -2.471 | (0.21) |
| Conqueror | -2.351 | (0.40) |
| Cordiale | -2.537 | (0.12) |
| Cougar | -2.323 | (0.45) |
| Crusoe | -2.591 | (0.06) |
| Delphi | -2.623 | (0.02) |
| Denman | -2.356 | (0.39) |
| Dickens | -2.111 | (0.95) |
| Duxford | -2.260 | (0.58) |

Table 6.5. Continued.

| Variety | Logit percentage of roots infected with take-all (back transformed means) | |
|---|---|---|
| Einstein | -2.540 | (0.12) |
| Evolution | -2.225 | (0.65) |
| Gallant | -2.518 | (0.15) |
| Grafton | -2.697 | (0) |
| Hereward | -2.206 | (0.70) |
| Horatio | -2.365 | (0.37) |
| Invicta | -2.539 | (0.12) |
| JB Diego | -2.368 | (0.37) |
| KWS Croft | -2.251 | (0.60) |
| KWS Gator | -2.403 | (0.31) |
| KWS Kielder | -2.250 | (0.60) |
| KWS Podium | -2.436 | (0.26) |
| KWS Santiago | -2.507 | (0.16) |
| KWS Sterling | -2.509 | (0.16) |
| KWS Target | -2.538 | (0.12) |
| Leeds | -2.615 | (0.03) |
| Monterey | -2.431 | (0.27) |
| Myriad | -2.286 | (0.52) |
| Panorama | -2.465 | (0.22) |
| Relay | -1.985 | (1.35) |
| Revelation | -2.364 | (0.38) |
| Scout | -2.421 | (0.28) |
| Solstice | -2.323 | (0.45) |
| Tuxedo | -2.221 | (0.66) |
| Viscount | -2.229 | (0.65) |
| Zulu | -2.463 | (0.22) |
| d.f. | 39 | |
| SED | 0.169 | |
| Wald statistic | 75.27 | |
| F probability | <0.001 | |
| Grand mean | -2.385 | (0.39) |

There were significant effects of variety on days to anthesis ($P$ = <0.001), days to leaf senescence ($P$ = <0.001), plant height ($P$ = <0.001) and plot yields ($P$ = <0.001) (Table 6.6). Due to the small plot size (1 m x 1 m) and the inherent edge effect, the varieties were ranked from the highest yield to the lowest yield (Table 6.6). The variety KWS Kielder had the highest recorded yield. A positive correlation was found between days to anthesis and days to leaf senescence, varieties that reached anthesis at an earlier date also went on to reach total leaf senescence at an earlier date (Figure 6.7). There was a weakly positive correlation between the percentage of roots colonised with *Phialophora* when baited on Hereward and days to anthesis (Hereward x days to anthesis: $r_s$ = 0.221, $P$ = 0.043, n = 40) and a moderately negative correlation between the percentage of roots colonised with *Phialophora* when baited with the field plot variety and days to anthesis (Field plot variety x days to anthesis: $r_s$ = -0.334, $P$ = 0.009, n = 40). There were no significant correlations between the percentage of roots colonised with *Phialophora* when baited on either Hereward or the plot variety and yield (Hereward x yield: $r_s$ = 0.102, $P$ = 0.133, n = 40; Field plot variety x yield: $r_s$ = -0.100, $P$ = 0.134, n = 40). Interestingly, the variety KWS Kielder had the highest yield across the field trial, yet a moderately high level of *Phialophora* colonisation when baited with either the variety Hereward or the field plot variety (Table 6.4 & Figure 6.4). There was a moderately positive correlation between the percentage of roots colonised with *Phialophora* when baited with Hereward and plant height (Hereward x days to plant height: $r_s$ = 0.343, $P$ = 0.008, n = 40) but no correlation between the percentage of roots colonised with *Phialophora* when baited with the field plot variety (Field plot variety x plant height: $r_s$ = -0.083, $P$ = 0.153, n = 40). There was a strong positive correlation between the percentage of roots colonised with *Phialophora* when baited with Hereward and days to leaf senescence ($r_s$ = 0.571, $P$ = <0.001, n = 40) (Figure 6.8) but no significant correlation was found when baited with the field plot variety ($r_s$ = -0.171, $P$ = 0.073, n = 40).

Table 6.6. Crop measurements of days to anthesis, days to leaf senescence, plant height and yields of the elite wheat varieties from field trial 2015/R/WW/1516.

| Variety | Days to anthesis (from 24/05/15) | Days to leaf senescence (from 18/07/15) | Plant height (cm) | Yield (tonnes/ha) | Ranking of yield from highest (1) to lowest (38) |
|---|---|---|---|---|---|
| Alchemy | 16.11 | 23.08 | 97.87 | 24.05 | 7 |
| Beluga | 13.53 | 15.93 | 85.21 | 21.21 | 32 |
| Cadenza | 13.13 | 18.93 | 106.98 | 20.48 | 34 |
| Chilton | 13.81 | 21.35 | 94.17 | 21.34 | 31 |
| Claire | 16.08 | 18.09 | 95.85 | 23.17 | 14 |
| Cocoon | 19.03 | 27.58 | 102.16 | 24.12 | 6 |
| Conqueror | 11.95 | 23.36 | 93.97 | 23.53 | 12 |
| Cordiale | 11.35 | 15.95 | 84.75 | 22.06 | 26 |
| Cougar | 15.94 | 25.73 | 91.79 | 22.67 | 23 |
| Crusoe | 12.12 | 19.45 | 84.7 | 24.76 | 3 |
| Delphi | 15.95 | 23.97 | 92.38 | 23.19 | 13 |
| Denman | 16.08 | 21.23 | 87.33 | 22.28 | 25 |
| Dickens | 11.53 | 22.96 | 88.51 | 23.13 | 15 |
| Duxford | 16.21 | 22.47 | 98.64 | 24.32 | 4 |
| Einstein | 9.67 | 18.28 | 90.32 | 22.95 | 17 |
| Evolution | 15.12 | 27.47 | 99.73 | 24.31 | 5 |
| Gallant | 9.12 | 9.95 | 87.07 | 22.05 | 27 |
| Grafton | 12.21 | 11.13 | 81.02 | 22.29 | 24 |
| Hereward | 13.81 | 22.45 | 91.91 | 19.08 | 36 |
| Horatio | 11.95 | 20.08 | 98.29 | 23.72 | 9 |
| Invicta | 12.69 | 25.54 | 94.83 | 23.64 | 11 |
| JB Diego | 11.81 | 21.71 | 95.24 | 22.84 | 20 |
| KWS Croft | 13.02 | 24.82 | 96.58 | 21.72 | 28 |
| KWS Gator | 12.03 | 20.06 | 91.99 | 24 | 8 |
| KWS Kielder | 15.96 | 24.31 | 92.32 | 25.57 | 1 |
| KWS Podium | 12.43 | 22.34 | 83.55 | 20.3 | 35 |
| KWS Santiago | 14.52 | 21.43 | 99.74 | 24.95 | 2 |
| KWS Sterling | 15.8 | 24.23 | 82.39 | 17.83 | 38 |
| KWS Target | 12.09 | 22.73 | 89.04 | 20.79 | 33 |
| Leeds | 15.3 | 25.19 | 94.26 | 23.09 | 16 |
| Monterey | 10.31 | 25.69 | 98.06 | 21.6 | 29 |
| Myriad | 16.01 | 23.82 | 103.55 | 22.71 | 21 |
| Panorama | 14.27 | 21.79 | 95.75 | 23.68 | 10 |
| Relay | 15.85 | 24.43 | 88.58 | 24.05 | 7 |
| Revelation | 16.33 | 26.76 | 91.38 | 22.7 | 22 |

Table 6.6. Continued.

| Variety | Days to anthesis (from 24/05/15) | Days to leaf senescence (from 18/07/15) | Plant height (cm) | Yield (tonnes/ha) | Ranking of yield from highest (1) to lowest (38) |
|---|---|---|---|---|---|
| Scout | 12.16 | 24.74 | 94.44 | 21.48 | 30 |
| Solstice | 12.03 | 18.44 | 96.15 | 18.3 | 37 |
| Tuxedo | 14.63 | 26.91 | 88.66 | 22.93 | 18 |
| Viscount | 12.9 | 24.2 | 89.23 | 22.88 | 19 |
| Zulu | 13.71 | 22.78 | 100.57 | 24 | 8 |
| d.f. | 39 | 39 | 39 | 39 | |
| SED | 0.74 | 1.226 | 1.308 | 0.824 | |
| F statistic | 17.76 | 22.42 | 44.39 | 9.69 | |
| F probability | <0.001 | <0.001 | <0.001 | <0.001 | |

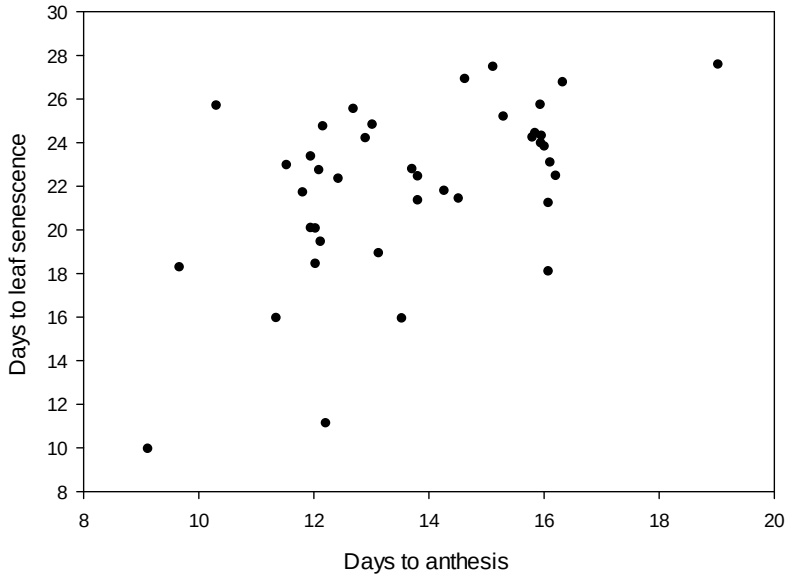



Figure 6.7. Correlation between days to anthesis (from 24/05/15) and days to leaf senescence (from 18/07/15) for field trial 2015/R/WW/1516. $r_s$ = 0.427, *P* = 0.001, n = 40.

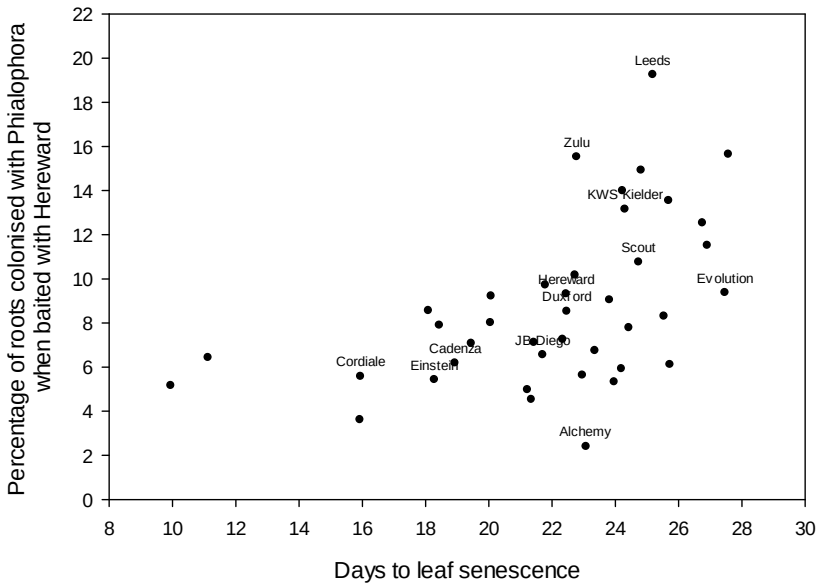



Figure 6.8. Correlation between days to leaf senescence (from 18/07/15) and percentage of roots colonised with *Phialophora* when baited with the variety Hereward for field trial 2015/R/WW/1516. $r_s$ = 0.571, *P* = <0.001, n = 40.

The above-ground phenotypic crop measurements were also correlated against the ranking of the year that each variety were first listed on the AHDB RL. No significant correlation was found between the ranked year first listed on RL and days to anthesis ($r_s$ = 0.208, *P* = 0.055, n = 40) and plant height ($r_s$ = 0.104, *P* = 0.141, n = 40). A strong positive correlation was however found between the ranked year first listed on RL and days to leaf senescence ($r_s$ = 0.537, *P* = <0.001, n = 40) (Figure 6.9). The pattern of the labelled varieties is similar to the pattern found for the correlation between the ranking of the year that the variety was first listed on the AHDB RL and the percentage of roots colonised with *Phialophora* when baited with the variety Hereward (Figure 6.6). The phenotypic crop measurements were then compared to the corresponding agronomic feature on the AHDB RL of 2013/2014. Days to anthesis was correlated against ripening (days +/- Solstice) data on the AHDB RL, a moderately positive correlation was found ($r_s$ = 0.485, *P* = <0.001, n = 38). Plant height was correlated against the plant height (cm) on the AHDB RL and a strong positive correlation was found ($r_s$ = 0.848, *P* = <0.001, n = 38). A moderately positive correlation was found between the field trial yield data and the grain yield as % control for UK on the AHDB RL ($r_s$ = 0.332, *P* = 0.011, n = 38).

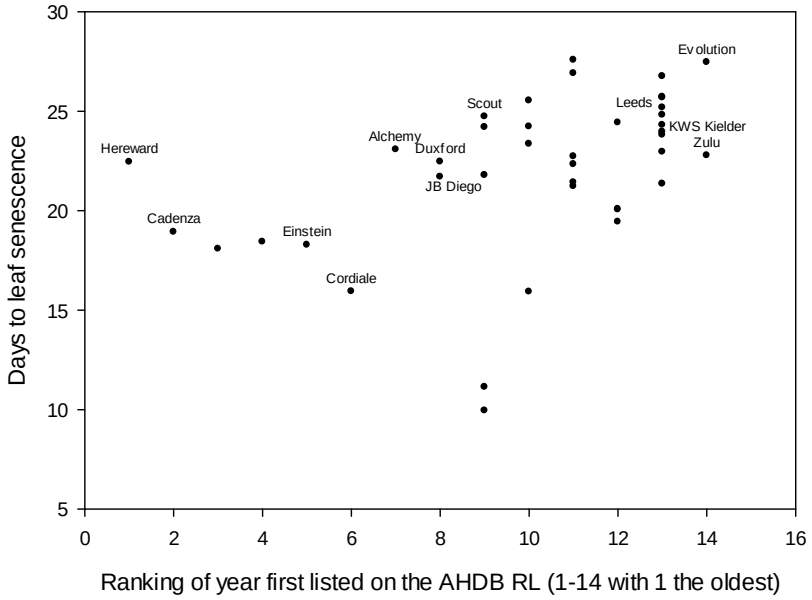



Figure 6.9. Correlation between days to leaf senescence (from 18/07/15) and the ranking of the year that the variety was first listed on the AHDB RL. $r_s$ = 0.537, P = <0.001, n = 40.

A subset of roots were examined under the light microscope and photographs taken to capture the sub-epidermal vesicles found within the roots from the *Phialophora* spp. colonisation (Figure 6.10 & Figure 6.11). Large single sub-epidermal vesicles, as seen in Figure 6.10, were found for the majority of colonised roots. Small clusters of sub-epidermal vesicles were occassionally identified on the colonised roots (Figure 6.11). *Phialophora* colonisation and take-all infection were found together on the same roots of pot bioassay 114.1C (plot Monterey and baited with Hereward) (Figure 6.12). *Phialophora* spp. were not isolated from these roots (see Table 6.7).

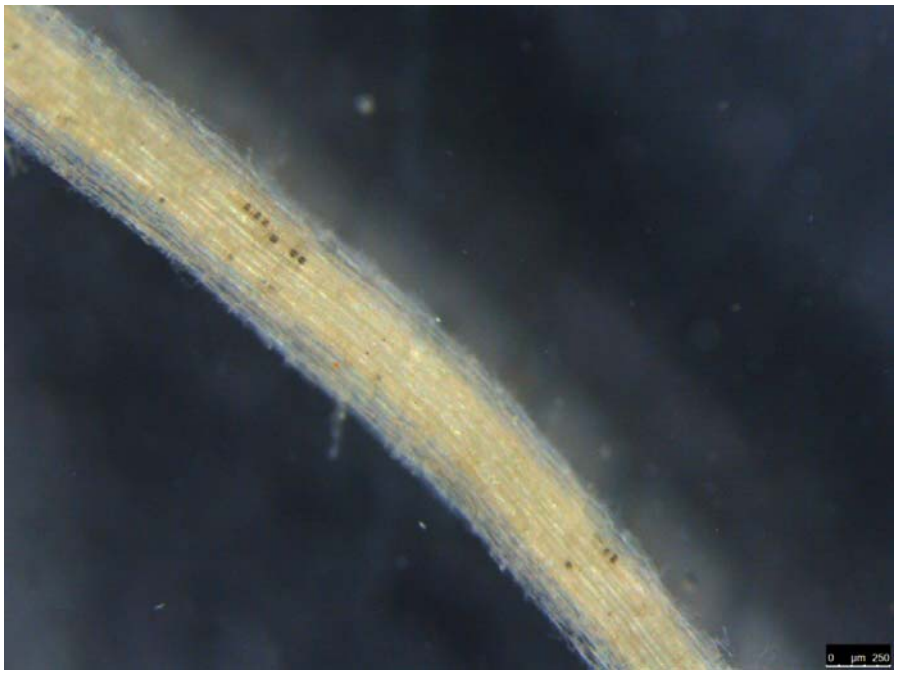


Figure 6.10. Microscopy image of *Phialophora* spp. colonised wheat seedling root from field plot 155, variety Revelation, and soil core bioassay pot 155.1A, baited with Hereward, of field trial 2015/R/WW/1516. Magnification X 41.7.

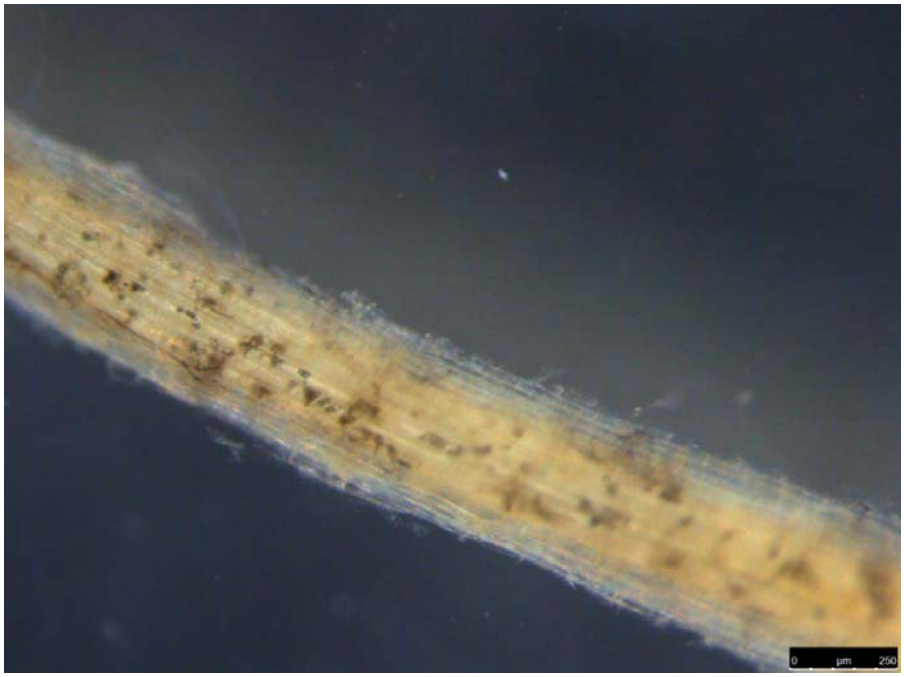


Figure 6.11. Microscopy image of *Phialophora* spp. colonised wheat seedling root from field plot 141, Duxford, and soil core bioassay pot 141.2A, baited with field plot variety Duxford, of field trial 2015/R/WW/1516. Magnification X 69.1

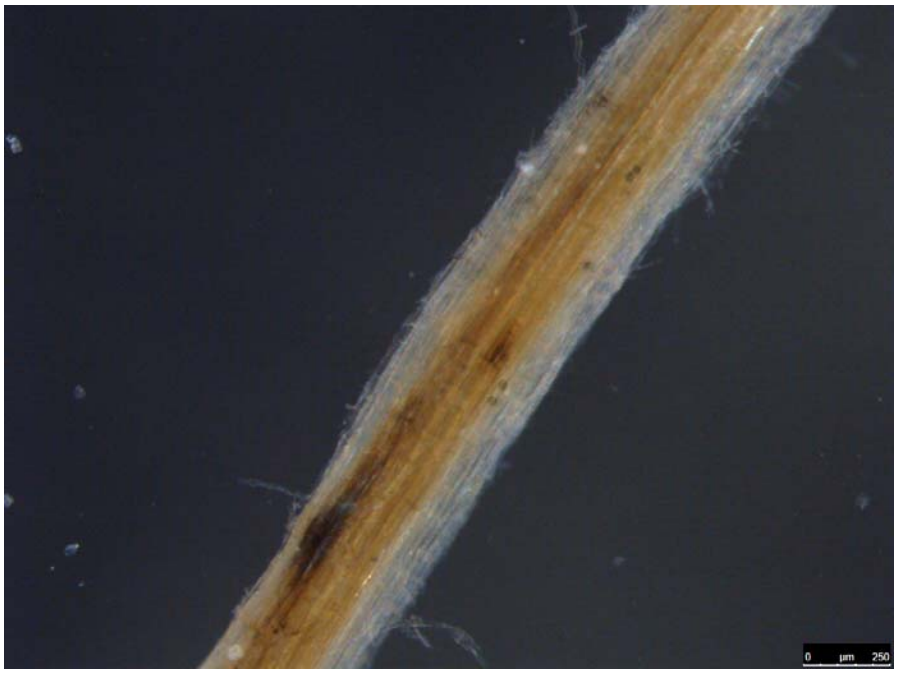



Figure 6.12. Microscopy image of *Phialophora* spp. colonised and take-all infected wheat seedling root from field plot 114, Monterey, and soil core bioassay pot 114.1C, baited with Hereward, of field trial 2015/R/WW/1516. Magnification X 56.6.

*Phialophora* spp. were isolated from root tissue of the soil core bioassay seedling wheat plants. DNA was extracted and a PCR performed to amplify ITS4 and ITS5 regions. Returned ITS sequences were then blasted in the NCBI BLAST tool for species identification (Table 6.7). Nineteen isolates were confirmed as *Phialophora* spp. (Table 6.7). The top two BLAST hits for all nineteen isolates were *Gaeumannomyces graminis* strains, which does not identify the isolates at the species level as *Gaeumannomyces graminis* does not distinguish between the take-all fungus and *Phialophora* species. It was therefore decided to take the third BLAST hit for each of the nineteen field isolates in order to get down to a species identification. Each of the nineteen isolates had the same two or three species hit for the third BLAST hit; *Ggg*, *Phialophora* sp. lobed hyphopodia and *Phialophora radicicola* (Table 6.7). The 523 bp region of the 19 ITS sequences were aligned in Clustal Omega (EMBL-EBI) and 5 single nucleotide polymorphisms (SNPs) were found between the first 17 bp on the 5' end and 4 SNPs were found between the last 19 bp of 3' of 523 bp region. This suggests that the 19 *Phialophora* spp. field

isolates are extremely similar and two of the top BLAST hits suggest that the isolates are *Phialophora* sp. lobed hyphopodia species. Interestingly, one of the hits is the *P. radicicola* species (protolog: *Phialophora zeicola* (Deacon and Scott, 1983), a *Phialophora* species that infects maize (Table 6.7).

Table 6.7. Species identification of field isolates from field trial 2015/R/WW/1516.

| Isolate ID | Field plot variety | Soil core bioassay bait (Hereward or field plot variety) | BLAST search | Total score | Query cover | Identification |
|---|---|---|---|---|---|---|
| NZ.16.1A.15 | Zulu | Hereward | *Gaeumannomyces graminis* var. *graminis*[1] | 917 | 95% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 917 | 95% | 99% |
| NZ.24.2A.15 | KWS Kielder | KWS Kielder | *Phialophora radicicola*[3] | 920 | 94% | 100% |
| | | | *Gaeumannomyces graminis* var. *graminis*[1] | 920 | 94% | 100% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 920 | 95% | 99% |
| NZ.112.1A.15 | KWS Target | Hereward | *Gaeumannomyces graminis* var. *graminis*[1] | 922 | 95% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 922 | 96% | 99% |
| NZ.136.1A.15 | Tuxedo | Hereward | *Phialophora radicicola*[3] | 913 | 95% | 99% |
| | | | *Gaeumannomyces graminis* var. *graminis*[1] | 913 | 95% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 913 | 95% | 99% |
| NZ.141.2A.15 | Duxford | Duxford | *Gaeumannomyces graminis* var. *graminis*[1] | 917 | 95% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 917 | 95% | 99% |
| NZ.155.1A.15 | Revelation | Hereward | *Phialophora radicicola*[3] | 920 | 94% | 100% |
| | | | *Gaeumannomyces graminis* var. *graminis*[1] | 920 | 94% | 100% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 920 | 95% | 99% |
| NZ.160.1A.15 | KWS Sterling | Hereward | *Phialophora radicicola*[3] | 913 | 94% | 99% |
| | | | *Gaeumannomyces graminis* var. *graminis*[1] | 913 | 94% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 913 | 95% | 99% |
| NZ.173.2A.15 | Solstice | Solstice | *Phialophora radicicola*[3] | 920 | 94% | 100% |
| | | | *Gaeumannomyces graminis* var. *graminis*[1] | 920 | 94% | 100% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 920 | 95% | 99% |
| NZ.8.1B.16 | Delphi | Hereward | *Gaeumannomyces graminis* var. *graminis*[1] | 922 | 95% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 922 | 95% | 99% |

Table 6.7. Continued.

| Isolate ID | Field plot variety | Soil core bioassay bait (Hereward or field plot variety) | BLAST search | Total score | Query cover | Identification |
|---|---|---|---|---|---|---|
| NZ.12.2B.16 | Solstice | Solstice | *Phialophora radicicola*[3] | 920 | 95% | 100% |
| | | | *Gaeumannomyces graminis* var. *graminis*[1] | 920 | 95% | 100% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 920 | 95% | 99% |
| NZ.110.2B.16 | Cordiale | Cordiale | *Gaeumannomyces graminis* var. *graminis*[1] | 922 | 95% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 922 | 96% | 99% |
| NZ.43.1C.16 | Relay | Hereward | *Phialophora radicicola*[3] | 913 | 94% | 99% |
| | | | *Gaeumannomyces graminis* var. *graminis*[1] | 913 | 94% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 913 | 95% | 99% |
| NZ.93.2C.16 | JB Diego | JB Diego | *Gaeumannomyces graminis* var. *graminis*[1] | 915 | 94% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 915 | 95% | 99% |
| NZ.103.2C.16 | Solstice | Solstice | *Phialophora radicicola*[3] | 913 | 95% | 99% |
| | | | *Gaeumannomyces graminis* var. *graminis*[1] | 913 | 95% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 913 | 95% | 99% |
| NZ.136.1C.2.16 | Tuxedo | Hereward | *Phialophora radicicola*[3] | 920 | 94% | 100% |
| | | | *Gaeumannomyces graminis* var. *graminis*[1] | 920 | 94% | 100% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 920 | 95% | 99% |
| NZ.138.1C.16 | Zulu | Hereward | *Phialophora radicicola*[3] | 913 | 95% | 99% |
| | | | *Gaeumannomyces graminis* var. *graminis*[1] | 913 | 95% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 913 | 95% | 99% |
| NZ.176.1C.16 | Evolution | Hereward | *Phialophora radicicola*[3] | 913 | 94% | 99% |
| | | | *Gaeumannomyces graminis* var. *graminis*[1] | 913 | 94% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 913 | 95% | 99% |

Table 6.7. Continued.

| Isolate ID | Field plot variety | Soil core bioassay bait (Hereward or field plot variety) | BLAST search | Total score | Query cover | Identification |
|---|---|---|---|---|---|---|
| NZ.183.1C.16 | Invicta | Hereward | *Gaeumannomyces graminis* var. *graminis*[1] | 918 | 95% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 918 | 95% | 99% |
| NZ.184.1C.16 | Monterey | Hereward | *Gaeumannomyces graminis* var. *graminis*[1] | 924 | 95% | 99% |
| | | | *Phialophora* sp. lobed hyphopodia[2] | 924 | 95% | 99% |

[1] *Gaeumannomyces graminis* var. *graminis* isolate 85/23-1 (NCBI accession: AJ010033.1) isolated from wheat at RRes in 1985 by R. J. Gutteridge (Ward and Gray, 1992).

[2] *Phialophora* sp. (lobed hyphopodia) isolate P2/P9 (NCBI accession: U17216.1). P2 (original name 1855) isolated from Czechoslovakia, source J. Henson. P9 (original name 89/5-3) isolated from wheat at RRes, source D. Hornby (Bryan et al., 1995).

[3] *Phialophora radicicola* (protolog: *Phialophora zeicola* (Deacon and Scott, 1983)) strain ATCC 28234 (NCBI accession: KM484850.1) isolated from maize in England (Klaubauf et al., 2014).

A phylogenetic tree was constructed to identify the genetic relationship between the *Phialophora* spp. field isolates from 2015/R/WW/1516 with the *Phialophora* field isolate collection gathered in Chapter 5 (see Table 5.7), as well as the relationship of the field isolates to reference *Phialophora* spp. and *Ggt* isolates submitted to the NCBI nucleotide database by a range of authors (see Appendix 6.2). Unfortunately, the cold room stock of *P. graminicola,* from the *Phialophora* isolate collection in Chapter 5, could not be included due to the extracted ITS region from the sequenced genome being too small. The *Phialophora* sp. (GP57) previously isolated at RRes (Ward and Bateman, 1999) and *Phialophora* sp. isolates K8 and 437 isolated in Germany (Ulrich et al., 2000) were included, as well as two *P. graminicola* isolates previously isolated at RRes (Bryan et al., 1995) and two *Ggg* (anamorph: *Phialophora* sp. lobed hyphopodia) sequences, one isolated from maize in China (W4066A) and one isolated from wheat at RRes (247) (Ward and Bateman, 1999). The data consisted of 77 aligned sequences of 19 ITS5-ITS4 rDNA regions of the field isolates from field trial 2015/R/WW/1516 and 9 ITS5-ITS4 rDNA regions of the *Phialophora* isolate collection detailed in Chapter 5 along with the five ITS sequences obtained from NCBI for the top three BLAST hits for the field isolates, along with 35 sequences of the take-all fungus (*Ggt*) as references, seven sequences of *Phialophora* spp. as references and two ITS sequences of *M. grisea* as an outlier group Figure 6.13 (see Appendix 6.2 for NCBI accessions).

As expected, the field isolates grouped together with the top BLAST hits but they were also found to group with a *Ggg* isolate and the *Phialophora* isolate collection gathered in Chapter 5, with the exception of isolate S.09.13 (Figure 6.13). The S.09.13 isolate, previously identified as an unnamed *Phialophora* sp. in Chapter 5, was found to be distinct from the field isolates and *Ggg* isolates and grouped with the three *Phialophora* sp. from the NCBI database with good support from bootstrapping. The two *P. graminicola* isolates grouped together, distinct from the *Phialophora* spp. field isolates and *Phialophora* sp., with high support from bootstrapping. Interestingly, the two *Ggg* isolates grouped separately from one another. The *Ggg* W4066A isolate, previously isolated from maize (Ward and Bateman, 1999), grouped separately from the *Ggg* 247 isolate, previously isolated

from wheat, which was found to group with the *Phialophora* field species that were also isolated from wheat. The *Ggt* isolates were found to group together with small distances between species. Within the *Ggt* group, three clusters formed. One of the clusters consisted of *Ggt* isolates AT2, AT3, GGTOR2 and haplotype S5. The *Ggt* isolates AT2, AT3 (Bryan et al., 1995) and haplotype S5 (Daval et al., 2010) have all been isolated from oats, the origin of host cannot be found for *Ggt* isolate GGTOR2. The other two clusters consisted of isolates originating from wheat, barley and rye.

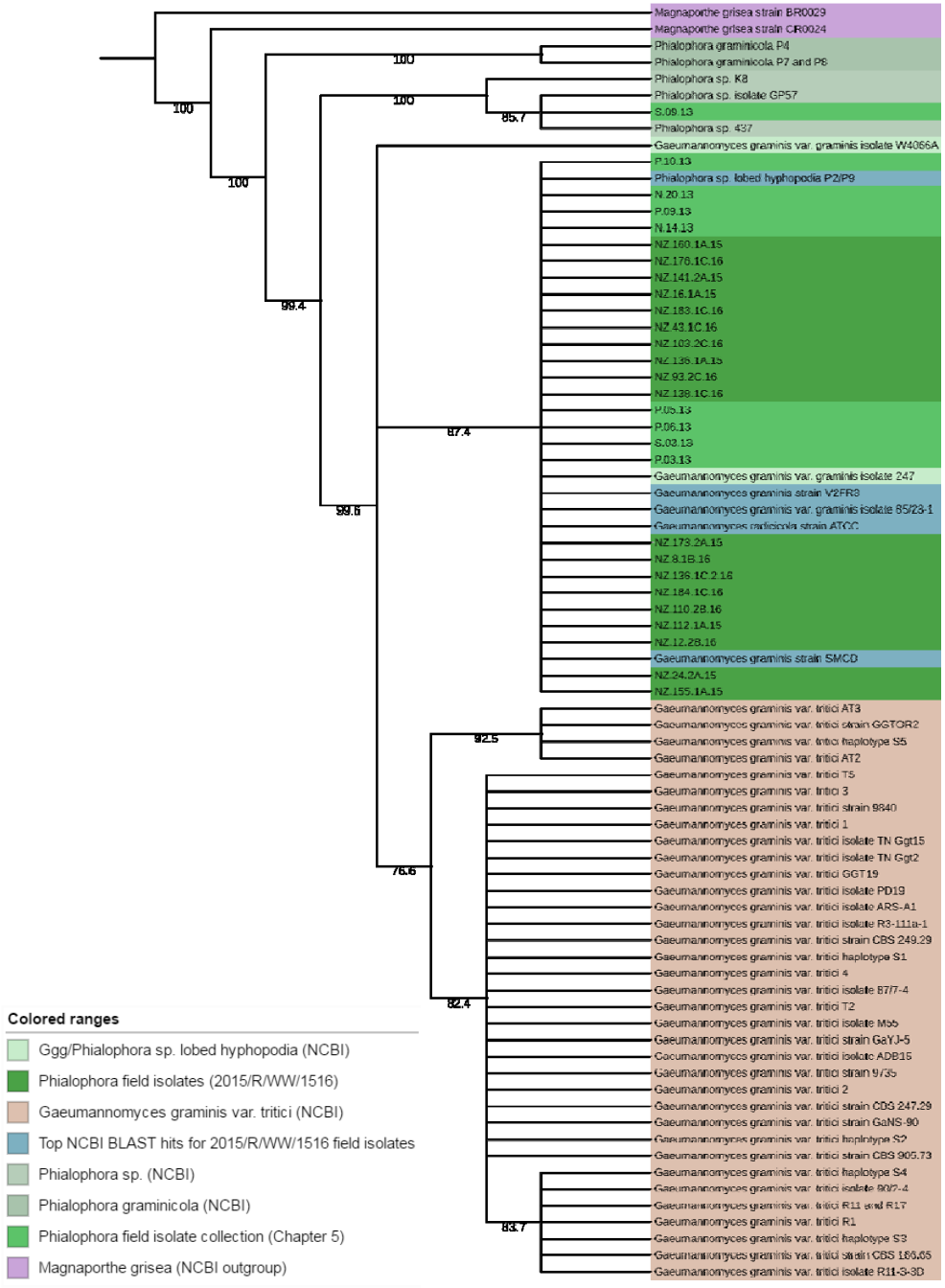



**Figure 6.13.** Phylogenetic tree of the ITS5- ITS4 rDNA regions of the field isolates and *Phialophora* isolate collection along with the ITS rDNA regions of the top three BLAST hits for the field isolates, and reference sequences for *Phialophora* spp., *Ggt* and the outlier group *M. grisea*. The genetic distance model Tamura-Nei was used and a tree build method of Neighbor-joining performed in Geneious. The values at the forks denote the number of times (percentage) in 1000 trees that this grouping occurred after the bootstrapping data. ITOL was used to illustrate the analysis.

### 6.3.2 Performance of additional cereal genotypes to *Phialophora* spp. in pot bioassays

A soil and sand calibration pot bioassay were carried out to test the best growth medium for *Phialophora* colonisation and to calibrate the sand maizemeal inoculum for both the *Phialophora* sp. lobed hyphopodia isolate (N.14.13) and *Phialophora* sp. isolate (S.09.13) (Table 6.8 & Table 6.9). Calibration of the *Phialophora* inoculum is required to develop a working pot bioassay in order to screen the ability of *Phialophora* spp. to colonise the roots of cereal genotypes. Seven sand maizemeal inoculum: silver sand dilutions (Table 6.8 & Table 6.9) were carried out to determine the dilution required to achieve ~50% of colonised *Phialophora* roots. A 50% level of *Phialophora* colonisation should allow good discrimination between cereal genotypes in the experimental pot bioassays.

The soil calibration pot bioassay produced a higher percentage of roots colonised with both *Phialophora* species (Table 6.8) than seen in the sand pot bioassay (Table 6.9). Yet, the required benchmark of ~50% of colonised *Phialophora* roots was only reached in the soil pot bioassay at a 1:50 dilution and when assessed only via the microscope. A main effect of dilution was significant with both assessment methods in the soil calibration pot bioassay, the percentage of *Phialophora* colonised roots increased as the sand maizemeal: silver sand dilution decreased (REML main effect of dilution with microscope assessment: $P$ = <0.001; REML main effect of dilution with visual assessment: $P$ = <0.001) (Table 6.8). A main effect of treatment was also significant across both assessment methods in the soil calibration pot bioassay, a higher level of colonised roots was observed when inoculated with the *Phialophora* sp. isolate across both assessments methods (REML main effect of treatment with microscope assessment: $P$ = 0.002; REML main effect of treatment with visual assessment: $P$ = <0.001). No significant interaction of dilution and treatment was found (REML microscope assessment dilution x treatment interaction: $P$ = 0.507, d.f. = 6, F statistic = 0.89, SED average = 0.257; REML visual assessment dilution x treatment interaction: $P$ = 0.162, d.f. = 1, F statistic = 1.61, SED average = 0.558). *Phialophora*-like species contamination was found from the soil in the negative

control of the soil pot bioassay. Microscopic analysis revealed small clustered sub-epidermal vesicles thought to be either *Phialophora graminicola* or *Phialophora* sp.

The ~50% percentage of roots colonised benchmark could not be reached with either *Phialophora* species in the sand calibration pot bioassay, regardless of the visual assessment method (Table 6.9). A main effect of dilution was only significant when the roots were assessed with the microscope, the percentage of *Phialophora* colonised roots increased as the sand maizemeal: silver sand dilution decreased (REML: $P$ = <0.001) (Table 6.9). A main effect of treatment was only significant when the roots were assessed by eye, a higher level of colonised roots was observed when inoculated with the *Phialophora* sp. (REML: $P$ = 0.009) (Table 6.9), as also observed in the soil calibration pot bioassay (Table 6.8). No significant interaction of dilution and treatment was found (REML microscope assessment dilution x treatment interaction: $P$ = 0.748, d.f. = 6, F statistic = 0.58, SED average = 0.343; REML visual assessment dilution x treatment interaction: $P$ = 0.986, d.f. = 1, F statistic = 0.16, SED average = 0.666). Overall, a low percentage of root area was colonised by the *Phialophora* species in the sand calibration pot bioassay and the sub-epidermal vesicles were sparse along the seedling roots. This made it particularly difficult to visualise when assessing by eye. The soil calibration pot bioassay method indicated a higher level of *Phialophora* spp. colonisation and was subsequently taken forward to screen the ability of *Phialophora* spp. to colonise the roots of cereal genotypes in experimental soil pot bioassays. The sand maizemeal: silver sand dilution would need to be reduced in order to assess roots in experimental soil pot bioassay by eye and not have to rely on the magnification of the microscope to visualise the sub-epidermal vesicles of *Phialophora* spp. colonisation.

Table 6.8. The ability of *Phialophora* spp. to colonise Hereward in a soil pot bioassay to calibrate sand maizemeal inoculum: silver sand dilutions.

| Soil pot bioassay calibration | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Microscope assessment** | | | | **Visual assessment** | | | |
| Sand maizemeal inoculum: silver sand dilution | Logit % of *Phialophora* colonised roots (back-transformed means) | Treatment | Logit % of *Phialophora* colonised roots (back-transformed means) | Sand maizemeal inoculum: silver sand dilution | Logit % of *Phialophora* colonised roots (back-transformed means) | Treatment | Logit % of *Phialophora* colonised roots (back-transformed means) |
| 0 | -2.015 (11.38) | Untreated | -2.015 (11.38) | 0 | -5.416 (0)[1] | Untreated | -5.416 (0)[1] |
| 1:50 | -0.003 (49.92) | *Phialophora* lobed | -0.837 (30.02) | 50 | -1.370 (19.96) | *Phialophora* lobed | -3.220 (3.38) |
| 1:100 | -0.422 (39.51) | *Phialophora* sp. | -0.566 (36.08) | 100 | -1.862 (13.08) | *Phialophora* sp. | -1.325 (20.71) |
| 1:150 | -0.448 (38.87) | d.f. | 1 | 150 | -1.815 (13.64) | d.f. | 1 |
| 1:200 | -0.588 (35.57) | SED average | 0.192 | 200 | -2.034 (11.18) | SED average | 0.410 |
| 1:250 | -1.090 (24.91) | F statistic | 8.11 | 250 | -2.983 (4.37) | F statistic | 83.85 |
| 1:300 | -1.140 (23.97) | F probability | 0.006 | 300 | -2.882 (4.86) | F probability | <0.001 |
| 1:350 | -1.221 (22.51) | | | 350 | -2.962 (4.47) | | |
| d.f. | 6 | | | d.f. | 6 | | |
| SED average | 0.199 | | | SED average | 0.431 | | |
| F statistic | 13.18 | | | F statistic | 5.79 | | |
| F probability | <0.001 | | | F probability | <0.001 | | |

[1] Back-transformation of the predicted logit mean of -5.416 is a negative number, this was assumed as 0 for analysis purposes.

Table 6.9. The ability of *Phialophora* spp. to colonise Hereward in a sand pot bioassay to calibrate sand maizemeal inoculum: silver sand dilutions.

| **Microscope assessment** | |
|---|---|
| Sand maizemeal inoculum: silver sand dilution | Logit % of *Phialophora* colonised roots (back-transformed means) |
| 0 | -5.245 (0.03)[1] |
| 50 | -0.682 (33.42) |
| 100 | -1.482 (18.20) |
| 150 | -1.672 (15.47) |
| 200 | -1.647 (15.81) |
| 250 | -1.904 (12.60) |
| 300 | -2.403 (7.88) |
| 350 | -2.148 (10.06) |
| d.f. | 6 |
| SED average | 0.266 |
| F statistic | 11.41 |
| F probability | <0.001 |

| **Visual assessment** | |
|---|---|
| Treatment | Logit % of *Phialophora* colonised roots (back-transformed means) |
| Untreated | -5.233 (0.04)[1] |
| *Phialophora* lobed | -4.641 (0.47) |
| *Phialophora* sp. | -3.565 (2.28) |
| d.f. | 1 |
| SED average | 0.491 |
| F statistic | 17.01 |
| F probability | 0.009 |

[1] Back-transformation of the predicted logit means of -5.245 and -5.233 can be assumed as 0 for analysis purposes as there were 0% of *Phialophora* colonised roots when assessed with the microscope and visually by eye.

An experimental pot bioassay was then carried out by AHDB summer bursary student Brittany Burton to screen the ability of *Phialophora* spp. to colonise the roots of selected cereal genotypes. Two inoculum methods were tested, a sand maizemeal *Phialophora* inoculum, as described in the calibration pot bioassays above, and *Phialophora* colonised potato dextrose agar (PDA), as described in Chapter 5. The soil calibration pot bioassay indicated that a sand maizemeal inoculum dilution of 1:50 provided the benchmark of 50% of *Phialophora* spp. colonised roots only when visualised under the microscope. A 1:50 sand maizemeal inoculum dilution provided only 20% of *Phialophora* spp. colonised roots when visually assessed by eye. The experimental pot bioassays were to be visually assessed by eye to reduce time and it was therefore decided that a lower inoculum dilution of 1:25 would be prepared for the *Phialophora* sp. isolate and that a 1:10 sand maizemeal dilution would be prepared for the *Phialophora* sp. lobed hyphopodia isolate, which indicated a much lower level of colonisation in the soil calibration pot bioassay. These lower dilutions were prepared in order to ensure that the 50% level of *Phialophora* spp. colonised roots was reached when visually assessing my eye. Due to the low level of *Phialophora* sp. lobed hyphopodia colonisation in the soil calibration pot bioassay, it was decided to also perform an experimental pot bioassay with the PDA inoculation method for both *Phialophora* spp. to establish whether this inoculation method provided a higher level of *Phialophora* root colonisation.

The sand maizemeal inoculation pot bioassay provided a significant interaction of the percentage of colonised roots between the two *Phialophora* spp. across the nine cereal genotypes (ANOVA *Phialophora* spp. x cereal genotype interaction: $P = <0.001$) (Table 6.10). The mean percentage of roots colonised by *Phialophora* sp. across the nine cereal genotypes ranged from 2.77%-62.84% and a smaller range of 0.96%-11.17% was found for roots colonised by *Phialophora* sp. lobed hyphopodia (Table 6.10). The percentage of roots colonised by *Phialophora* sp. was higher than *Phialophora* sp. lobed hyphopodia for all cereal genotypes, except in rye (Table 6.9). A significant interaction of the number of colonised roots between the two *Phialophora* spp. across the nine cereal genotypes was also found (ANOVA

*Phialophora* spp. x cereal genotype interaction: *P* = <0.001) (Table 6.10). *Phialophora*-like species were found on the roots of the Hereward negative controls, equating to less than one root per plant (Table 6.10). A significant difference in the total number of roots per plant across the cereal genotypes was only found for *Phialophora* spp. (ANOVA: *P* = <0.001) (Table 6.10), with rye and triticale having the highest number of roots and Hereward and the *T. monococcum* accession MDR046. There was also no correlation found between the number of roots per plant and the percentage of roots colonised by either *Phialophora* sp. lobed hyphopodia ($r_s$ = -0.317, *P* = 0.096, n = 9) or *Phialophora* sp. ($r_s$ = 0.050, *P* = 0.220, n = 9).

The PDA inoculation pot bioassay provided a higher level of *Phialophora* colonisation for both *Phialophora* spp. compared to the sand maizemeal inoculum pot bioassay. A grand mean of 24.86% *Phialophora* sp. lobed hyphopodia colonised roots was found in the PDA inoculum pot bioassay compared to only 5.94% of colonised roots in the sand maizemeal inoculum pot bioassay (Table 6.10 & Table 6.11), along with a grand mean of 29.13% *Phialophora* sp. colonised roots in the PDA inoculum pot bioassay compared to a slightly lower grand mean of 26.29% of colonised roots in the sand maizemeal inoculum pot bioassay (Table 6.10 & Table 6.11). The PDA inoculum pot bioassay provided a significant interaction of the percentage of colonised roots between the two *Phialophora* spp. across the nine cereal genotypes (ANOVA *Phialophora* spp. x cereal genotype interaction: *P* = <0.001) (Table 6.11). The mean percentage of roots colonised by *Phialophora* sp. across the nine cereal genotypes ranged from 4.33%-58.32% and a slightly smaller range of 1.23% - 44.15% was found for roots colonised by *Phialophora* sp. lobed hyphopodia (Table 6.11). Again, as seen in the sand maizemeal inoculum pot bioassay, the percentage of roots colonised by *Phialophora* sp. was higher than *Phialophora* sp. lobed hyphopodia for all cereal genotypes, except in rye. A significant interaction of the number of colonised roots between the two *Phialophora* spp. across the nine cereal genotypes was also found (ANOVA *Phialophora* spp. x cereal genotype interaction: *P* = <0.001) (Table 6.11). *Phialophora*-like colonisation was again found on less than one root per plant of the

Hereward negative control and microscopic analysis revealed the *Phialophora*-like species produced small clustered sub-epidermal vesicles and is therefore thought to be either *Phialophora graminicola* or *Phialophora* sp. (Figure 6.14). A significant difference in the total number of roots per plant across the cereal genotypes was found for *Phialophora* spp. (ANOVA: $P$ = <0.001) (Table 6.12), with the same trend as the sand maizemeal inoculum pot bioassay of rye and triticale having the highest number of roots and Hereward and the *T. monococcum* accession MDR046 (Table 6.12). A significant difference in the total number of roots per plant between the two *Phialophora* spp. was also found with plants inoculated with *Phialophora* sp. producing a higher number of roots (ANOVA: $P$ = 0.034) (Table 6.12). There was also no correlation found between the number of roots per plant and the percentage of roots colonised by either *Phialophora* sp. lobed hyphopodia ($r_s$ = 0.233, $P$ = 0.130, n = 9) or *Phialophora* sp. ($r_s$ = 0.033, $P$ = 0.228, n = 9). The PDA experimental pot bioassay was repeated in December 2014 and indicated similar patterns to the first PDA pot bioassay (Table 6.11, Table 6.12 & Table 6.13). A slightly lower grand mean of *Phialophora* sp. lobed colonised roots was found compared to the first PDA pot bioassay but again, as seen in both the sand maizemeal inoculum and PDA inoculum pot bioassay, the percentage of roots colonised by *Phialophora sp.* was higher than *Phialophora* sp. lobed hyphopodia for all cereal genotypes, except in rye (Table 6.13).

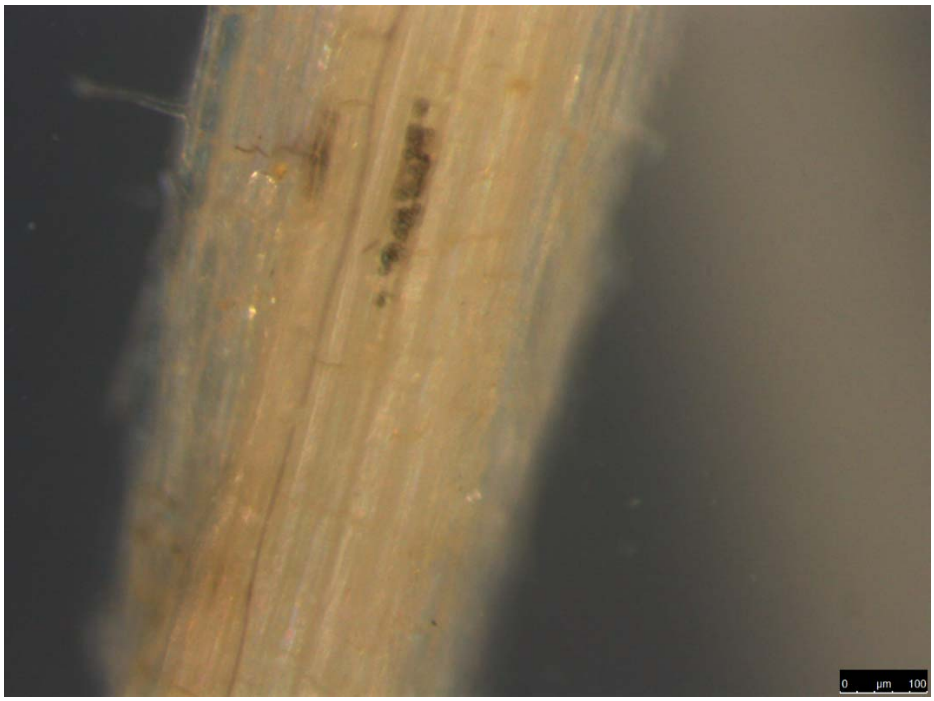



**Figure 6.14.** Microscopy image of *Phialophora*-like species on root from Hereward negative control with non-colonised PDA pot. Magnification X 16.

Table 6.10. The ability of *Phialophora* spp. to colonise the roots of cereal genotypes in a sand maizemeal inoculated pot bioassay in soil.

| Cereal genotype | ***Phialophora* sp. lobed hyphopodia** | | | ***Phialophora* sp.** | | | ***Phialophora* spp.[2]** |
|---|---|---|---|---|---|---|---|
| | **Logit percentage of colonised roots (back-transformed means)** | | **Number of colonised roots per plant** | **Logit percentage of colonised roots (back-transformed means)** | | **Number of colonised roots per plant** | **Number of roots per plant** |
| Oats | -4.22 | (0.96) | 0.11 | -2.17 | (9.83) | 0.85 | 8.33 |
| Rye | -2.37 | (8.16) | 1.02 | -3.40 | (2.77) | 0.35 | 11.90 |
| Triticale | -2.04 | (11.17) | 1.46 | -0.52 | (37.23) | 4.54 | 11.87 |
| Hereward | -2.20 | (9.56) | 0.67 | 0.52 | (62.84) | 3.72 | 6.26 |
| Hereward -[1] | -2.70 | (5.87) | 0.40 | -2.47 | (7.40) | 0.51 | 5.77 |
| Paragon | -2.49 | (7.24) | 0.63 | -0.57 | (36.02) | 2.86 | 8.30 |
| Watkins 777 | -3.25 | (3.28) | 0.33 | -0.37 | (40.74) | 3.83 | 9.47 |
| *T. m* MDR037 | -2.23 | (9.33) | 0.85 | 0.20 | (54.90) | 4.09 | 7.74 |
| *T. m* MDR046 | -2.70 | (5.85) | 0.38 | -0.40 | (40.07) | 2.72 | 6.28 |
| d.f. | 8 | | 8 | 8 | | 8 | 8 |
| SED | 0.428 | | 0.336 | 0.428 | | 0.336 | 0.497 |
| F Probability | <0.001 | | <0.001 | <0.001 | | <0.001 | <0.001 |
| Grand mean | -2.69 | (5.94) | 0.65 | -1.02 | (26.29) | 2.61 | 8.43 |

[1] Hereward - = Hereward negative control with silver sand.

[2] *Phialophora* spp. = *Phialophora* sp. and *Phialophora* sp. lobed hyphopodia.

Table 6.11. The ability of *Phialophora* spp. to colonise the roots of cereal genotypes in a PDA inoculated pot bioassay in soil.

| Cereal genotype | ***Phialophora* sp. lobed hyphopodia** | | | ***Phialophora* sp.** | | |
|---|---|---|---|---|---|---|
| | **Logit percentage of colonised roots (back-transformed means)** | | **Number of colonised roots per plant** | **Logit percentage of colonised roots (back-transformed means)** | | **Number of colonised roots per plant** |
| Oats | -4.05 | (1.23) | 0.15 | -2.72 | (5.75) | 0.53 |
| Rye | -0.88 | (29.17) | 2.96 | -2.99 | (4.33) | 0.47 |
| Triticale | -0.26 | (43.56) | 4.47 | -0.01 | (49.76) | 5.75 |
| Hereward | -0.41 | (39.82) | 2.42 | 0.03 | (50.69) | 3.00 |
| Hereward -[1] | -2.12 | (10.29) | 0.67 | -1.85 | (13.22) | 0.87 |
| Paragon | -0.83 | (30.14) | 2.40 | -0.65 | (34.16) | 2.77 |
| Watkins 777 | -0.37 | (40.75) | 3.50 | 0.09 | (52.34) | 4.77 |
| *T. m* MDR037 | -0.68 | (33.42) | 2.81 | 0.33 | (58.32) | 4.16 |
| *T. m* MDR046 | -0.23 | (44.15) | 2.81 | -0.15 | (46.35) | 3.33 |
| d.f. | 8 | | 8 | 8 | | 8 |
| SED | 0.455 | | 0.568 | 0.46 | | 0.6 |
| F Probability | <0.001 | | <0.001 | <0.001 | | <0.001 |
| Grand mean | -1.09 | (24.86) | 2.47 | -0.88 | (29.13) | 2.9 |

[1] Hereward - = Hereward negative control with non-colonised PDA.

Table 6.12. Number of roots per plant in a PDA inoculated pot bioassay in soil.

| | ***Phialophora* spp.**[2] | | |
|---|---|---|---|
| **Cereal genotype** | **Number of roots per plant** | ***Phialophora* spp.** | **Number of roots per plant** |
| Oats | 7.60 | *Phialophora* sp. lobed hyphopodia | 7.78 |
| Rye | 10.32 | *Phialophora* sp. | 8.17 |
| Triticale | 10.91 | d.f. | 1 |
| Hereward | 5.97 | SED | 0.177 |
| Hereward -[1] | 5.87 | F Probability | 0.034 |
| Paragon | 7.88 | Grand mean | 7.98 |
| Watkins 777 | 8.77 | | |
| *T. m* MDR037 | 7.66 | | |
| *T. m* MDR046 | 6.84 | | |
| d.f. | 8 | | |
| SED | 0.376 | | |
| F Probability | <0.001 | | |
| Grand mean | 7.98 | | |

[1] Hereward - = Hereward negative control with non-colonised PDA.

[2] *Phialophora* spp. = *Phialophora* sp. and *Phialophora* sp. lobed hyphopodia.

Table 6.13. The ability of *Phialophora* spp. to colonise the roots of cereal genotypes in a repeat PDA inoculated pot bioassay.

| Cereal genotype | ***Phialophora* sp. lobed hyphopodia** | | | ***Phialophora* sp.** | | | ***Phialophora* spp.** [2] |
|---|---|---|---|---|---|---|---|
| | **Logit percentage of colonised roots (back-transformed means)** | | **Number of colonised roots per plant** | **Logit percentage of colonised roots (back-transformed means)** | | **Number of colonised roots per plant** | **Number of roots per plant** |
| Oats | -3.68 | (1.99) | 0.17 | -2.40 | (7.89) | 0.86 | 7.97 |
| Rye | -1.11 | (24.48) | 2.40 | -2.63 | (6.27) | 0.76 | 10.21 |
| Triticale | -1.42 | (19.22) | 2.46 | -0.06 | (48.61) | 5.89 | 11.79 |
| Hereward | -0.37 | (40.79) | 2.71 | 0.41 | (60.11) | 3.76 | 6.44 |
| Hereward -[1] | -5.30 | (0) | 0 | -4.37 | (0.76) | 0.07 | 6.65 |
| Paragon | -1.03 | (26.68) | 2.03 | 0.24 | (57.35) | 4.37 | 7.78 |
| Watkins 777 | -0.10 | (48.13) | 2.67 | 0.29 | (66.20) | 5.07 | 9.07 |
| *T. m* MDR037 | -0.07 | (33.30) | 3.77 | 0.67 | (53.28) | 5.13 | 7.83 |
| *T. m* MDR046 | -0.69 | (26.05) | 2.33 | 0.13 | (56.13) | 3.74 | 6.95 |
| d.f. | 8 | | 8 | 8 | | 8 | 8 |
| SED | 0.481 | | 0.613 | 0.481 | | 0.613 | 0.430 |
| F Probability | 0.005 | | <0.001 | 0.005 | | <0.001 | <0.001 |
| Grand mean | -1.63 | (16.05) | 2.06 | -0.858 | (29.57) | 3.29 | 8.30 |

[1] Hereward - = Hereward negative control with non-colonised PDA.

[2] *Phialophora* spp. = *Phialophora* sp. and *Phialophora* sp. lobed hyphopodia.

A pooled REML variance components analysis was performed to combine the two experimental pot bioassays testing the sand maizemeal inoculation method and the PDA inoculation method, carried out by AHDB summer bursary student Brittany Burton, along with the repeat pot bioassay of the PDA inoculation method. A significant interaction for the percentage of colonised roots was found between the two *Phialophora* species for the nine cereal genotypes in all three experimental pot bioassays (REML *Phialophora* species x cereal genotype interaction: $P = <0.001$) (Table 6.14). A higher percentage of roots was colonised by *Phialophora* sp. across all nine cereal genotypes with a mean percentage of colonised roots ranging from 4.81%-59.76% (Table 6.14) compared to a smaller range of 1.77%-31.04% found for roots colonised by *Phialophora* sp. lobed hyphopodia (Table 6.14). A number of trends could be seen across the combined experimental pot bioassays. The *T. monococcum* accession MDR037 indicated the highest level of colonisation by both *Phialophora* species across all three experimental pot bioassays (Table 6.14). Oats indicated the lowest level of colonisation by *Phialophora* sp. lobed hyphopodia and a relatively low level of colonisation was also seen by *Phialophora* sp. In addition, a low level of *Phialophora* colonisation was also found in the negative control variety Hereward as discussed above. Overall, the percentage of roots colonised by *Phialophora* sp. was higher than the percentage of colonised roots by *Phialophora* sp. lobed hyphopodia across all cereal genotypes, except in rye, for all three experimental pot bioassays.

Table 6.14. Combined analysis of the ability of *Phialophora* spp. to colonise the roots of cereal genotypes in the sand maizemeal inoculum pot bioassay and the two PDA inoculum pot bioassays.

| | ***Phialophora* sp. lobed hyphopodia** | ***Phialophora* sp.** |
|---|---|---|
| **Cereal genotype** | **Percentage of colonised roots** | **Percentage of colonised roots** |
| Oats | 1.77 | 9.21 |
| Rye | 21.15 | 4.81 |
| Triticale | 25.90 | 45.26 |
| Hereward + | 30.31 | 57.81 |
| Hereward - | 6.05 | 8.39 |
| Paragon | 21.80 | 42.04 |
| Watkins line 777 | 24.66 | 50.16 |
| *T. m* MDR037 | 31.04 | 59.76 |
| *T. m* MDR046 | 28.36 | 46.70 |
| d.f. | 8 | |
| SED | 4.917 | |
| F statistic | 8.89 | |
| F probability | <0.001 | |
| Grand mean | 21.23 | 36.02 |

## 6.4 Discussion

### 6.4.1 Field trials

A field trial was carried out to evaluate the ability of natural populations of *Phialophora* fungi to colonise the roots of elite wheat varieties and the ability of the elite wheat varieties to build-up *Phialophora* species in the soil during a first wheat. *Phialophora* spp. could not be easily visually identified on the adult plant root systems and so these were subsequently omitted from the study. Ten plots were visually assessed under a light microscope for *Phialophora* colonisation and sub-epidermal vesicles were only visible on the seminal roots of one plant. Identification methods for adult plants were discussed previously in the chapter (eg. staining methods), yet it was decided that due to time constraints these methods would not be trialled. Post-harvest soil cores were then taken to measure the amount of *Phialophora* inoculum that had built up under the elite wheat first wheat. A range in wheat varietal ability to build-up *Phialophora* species was found. This study has found that overall, Hereward was a good bait variety for *Phialophora* colonisation. A higher level of *Phialophora* colonisation was found when soil cores were baited with Hereward rather than the field plot variety for the majority of elite wheat varieties. However, an interaction was found between the baiting methods, which suggests that we cannot only use Hereward as a general bait variety when studying the build-up of *Phialophora* in a first wheat. Hereward is typically used as a bait in soil core bioassays when evaluating the ability of wheat varieties to build-up take-all inoculum under a first wheat crop (McMillan et al., 2011). When the build-up of *Phialophora* is assessed in future field trial experiments, the field plot variety will need to be included as the bait for the soil core bioassays.

A subset of elite wheat varieties indicated the ability to build-up *Phialophora* species. Elite varieties such as JB Diego, Scout and KWS Kielder have the potential to maintain populations of *Phialophora*, whereas varieties such as Alchemy and Beluga would not. In particular, the elite varieties Scout and KWS Kielder indicate the highest level of *Phialophora* spp. colonisation independent of baiting method. It would be these varieties that would be best to build-up *Phialophora* fungi in the farmer's soil as their ability to build-up *Phialophora* inoculum would not be

dependent on having to grow the same first and second wheat variety, since the majority of farmers grow a different second wheat variety compared to the first wheat variety. Although the AHDB 2013/2014 RL was screened, varieties Scout and KWS Kielder that indicate the highest levels of *Phialophora* colonisation, independent of baiting variety, are all still on the AHDB RL for 2016/2017. There does not appear to be any interaction between *Phialophora* colonisation of the roots and the nabim groups of the elite wheat varieties. There also does not appear to be a particularly strong relationship between *Phialophora* colonisation and the year that the wheat varieties were first listed on the AHDB RL. A weakly negative correlation was found between the year that varieties were first listed on the AHDB RL and the percentage of roots colonised with *Phialophora* when baited with the plot variety and a moderately positive correlation was found between the year that the varieties were first listed on the RL and the percentage of roots colonised with *Phialophora* when baited with the variety Hereward. However, there were only a few varieties screened that were first listed in the earlier years compared to the later years of 2011 through to 2013. There are a number of varieties that have indicated a higher level of *Phialophora* colonisation than the older varieties of Cadenza and Hereward, which suggests that recent breeding has not reduced the ability of *Phialophora* species to colonise winter wheat varieties.

Microscopy analysis of the wheat seedling roots of the baited post-harvest soil cores revealed that two *Phialophora* spp. were present in the soil of the field site. The majority of roots illustrated large sub-epidermal vesicles and were visually identified as *Phialophora* sp. lobed hyphopodia. Small clustered sub-epidermal vesicles were occasionally found on roots, suggesting that either *Phialophora* sp. or *P. graminicola* were present in the field area. Some roots exhibited both *Phialophora* colonisation and take-all infection. This suggests that in these areas of the field site, there were either low levels of *Phialophora* populations or that the *Phialophora* fungi was not able to colonise the root tissue before the *Ggt* fungus in order to suppress take-all. Nineteen *Phialophora* spp. isolates were isolated from the wheat seedling roots from the baited post-harvest soil cores. The ITS sequence analysis revealed that the three BLAST hits for species identification were

*Phialophora* sp. lobed hyphopodia and the *Phialophora* sp. lobed hyphopodia teleomorph *Ggg*, and *P. radicicola*/*P. zeicola* (Deacon and Scott, 1983). This analysis suggested that the 19 field isolates were *Phialophora* sp. lobed hyphopodia and that no *Phialophora* sp./*P. graminicola*, previously identified from the visual microscopy analysis, were isolated. It is unclear why *P. radicicola*/*P. zeicola,* a species that infects maize, was identified as one of the top BLAST hits for the field isolates. It may be that ITS sequence analysis is not enough to differentiate between these *Phialophora* species.

A phylogenetic tree was constructed to identify the genetic relationship of the field isolates from field trial 2015/R/WW/1516 with the *Phialophora* isolate collection from Chapter 5, as well as against a number of *Phialophora* spp. and *Ggt* reference sequences from the NCBI nucleotide database. The phylogenetic analysis of the ITS sequences suggested that the field isolates from this chapter are the same species, *Phialophora* sp. lobed hyphopodia, as the *Phialophora* isolate collection (excluding isolate S.09.13 and the *P. graminicola* isolate). The *Phialophora* isolate collection S.09.13 isolate, identified as the unnamed *Phialophora* sp., formed a distinct group with the *Phialophora* sp. isolates from the Ward and Bateman (1999) and Ulrich et al. (2000) studies. This corresponds to a recent phylogenetic analysis by Hernández-Restrepo et al. (2016) that found that the unnamed *Phialophora* sp. isolated in the Ward and Bateman (1999) was found to represent a distinct lineage within *Magnaporthaceae*. The *P. graminicola* sequences downloaded from the NCBI nucleotide database were also found to form a distinct group (Hernández-Restrepo et al., 2016).

As discussed previously, *Phialophora* inoculum does not appear to persist when soil is artificially inoculated (Wong and Southwell, 1980, Martyniuk and Myskow, 1984, Gutteridge et al., 2007). It would be interesting to take isolated *Phialophora* species from surrounding soil of the elite wheat varieties that indicated the highest level of *Phialophora* colonisation from our field trial. These isolated *Phialophora* species could then be put back into the soil to inoculate the site. This form of artificial inoculation could help to reduce the breakdown of inoculum found in previous field trials (Wong and Southwell, 1980, Martyniuk and Myskow, 1984, Gutteridge et al.,

2007). The isolated *Phialophora* species could also be used to test the performance of a seed coat dressing. It would be important to thoroughly test that no adverse effects occurred on wheat plant growth or crop yield from the colonisation of these *Phialophora* spp.

McMillan et al. (2011) found similarities between closely related wheat varieties in their ability to build-up take-all inoculum. For example Cadenza was a low *Ggt* building variety and the Cadenza pedigree was also found in Cordiale and Xi19, both low to medium building varieties themselves (McMillan et al., 2011). The same trend is not found with ability of elite wheat varieties to build-up *Phialophora* inoculum. Hereward was suggested to be a medium builder of *Phialophora* inoculum, whereas the variety is a high builder of take-all inoculum (McMillan et al., 2011) and is highly susceptible to take-all infection. Cadenza and Cordiale were found to have similar levels of *Phialophora* colonisation when baited with Hereward but Cordiale exhibited a higher level of *Phialophora* colonisation than Cadenza when these varieties were baited back onto themselves in a second wheat situation. The Cordiale pedigree is found in the varieties Crusoe, Grafton and KWS Sterling, yet these varieties all have extremely low levels of *Phialophora* colonisation when baited back on to the field plot. The Robigus pedigree is also found in a number of varieties within the AHDB 2013/2014 RL and lines from the 2014/2015 RL winter wheat varieties, yet there appears to be no similarities across these varieties in their level of *Phialophora* colonisation with either baiting method. There appears to be more variation in *Phialophora* colonisation when baited with Hereward. The variation in *Phialophora* colonisation across the AHDB RL suggests that the ability to build-up *Phialophora* populations is not clearly associated with parentage and could also be influenced by a number of environmental factors such as soil type, soil moisture and the rhizosphere/soil microbiome.

During 2012, *Phialophora* species compromised a field trial in the New Zealand field (2012/R/CS/726) (see Figure 6.15) that was conducted by Vanessa McMillan. The field trial was exploring the ability of wheat cultivars to build-up take-all inoculum. Soil cores were taken from the field trial and a soil bioassay was carried out. The percentage of roots infected with take-all varied across the field site. The southern

two thirds of the trial site had only 7.5% roots infected with take-all compared to 36.9% of roots infected with take-all in the northern third of the trial (Vanessa McMillan, personal communication). *Phialophora* colonisation was found on the roots in the southern two thirds of the trial and it was thought that the presence of *Phialophora* fungi was suppressing the levels of take-all infection in this part of the field trial. *Phialophora* fungi were subsequently isolated from soil collected from the southern two thirds of the 2012/R/CS/726 field site in 2013 during this PhD and these *Phialophora* species form part of the *Phialophora* isolate collection that is detailed in Chapter 5. Due to the presence of *Phialophora* fungi, the southern two thirds of the 2012/R/CS/726 field site in the south west corner of the New Zealand field was therefore chosen to conduct the two *Phialophora* field trials 2015/R/WW/1516 and 2016/R/WW/1620 as part of this PhD project (illustrated as SW 1 in Figure 6.15). The south west 1 (SW 1) corner of the New Zealand field was originally the site of the Rothamsted Manor Kitchen Garden and this corner of the field has differing soil properties to the remainder of the field (Figure 6.15). The New Zealand field was in permanent grassland from 1929-1979 (Johnston et al., 1980). Previous studies in 1979 and 1999 found that the soil in the south west corner had a higher level of plant available phosphorus and exchangeable potassium than the rest of the sampled field (Figure 6.15) (Andy Macdonald, personal communication). The pH has also been found to be higher in the south west corner compared to the remainder of the field (Figure 6.15) (Andy Macdonald, personal communication). The soil carbon content was also calculated in the 1979 study and was found to be higher in the south west corner than elsewhere in the field (SW 1 = 4.059 %C, NW 1 = 3.195 %C and E 2 = 3.327 %C) (Andy Macdonald, personal communication). There is not enough known about the effects of different soil properties on the ability to sustain *Phialophora* populations and little information on the population dynamics of *Phialophora* spp. in wheat fields (Gutteridge et al., 2007). It can be seen that the Rothamsted Manor Kitchen Garden has altered the soil properties of the site in the New Zealand field compared to the remainder of the field and may have subsequently influenced the microbiota of the soil in this region, in particular sustaining populations of *Phialophora* spp.

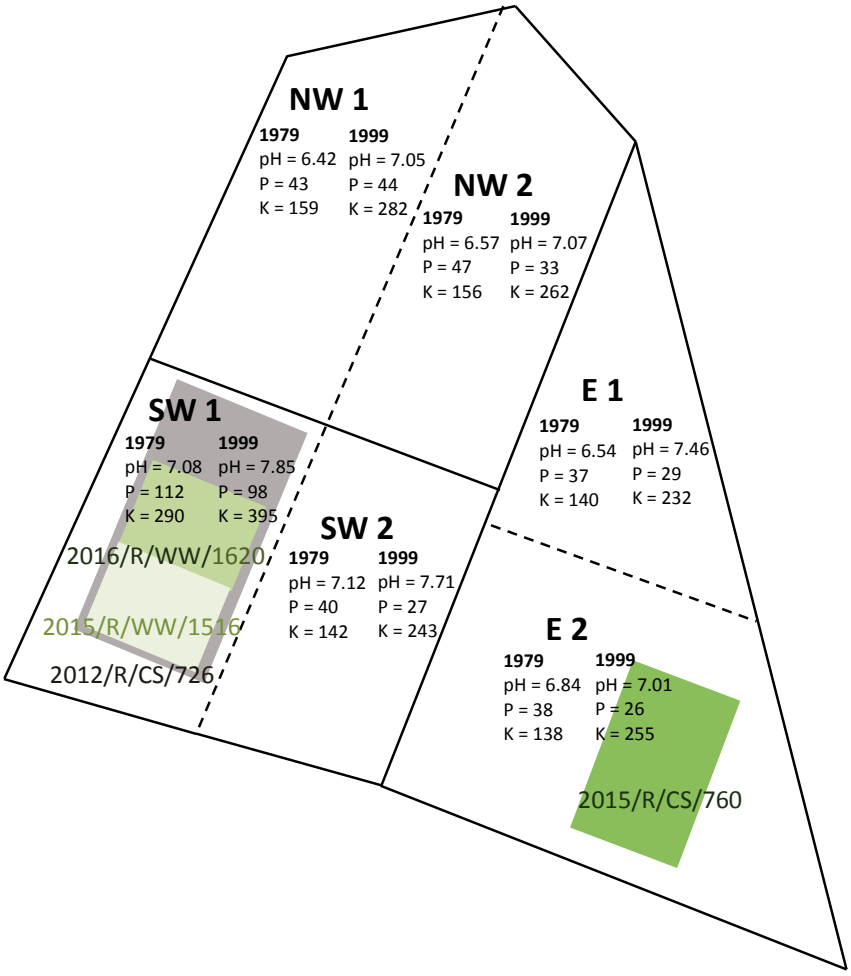



Figure 6.15. Adapted illustration from Andy MacDonald, RRes, of the soil properties collected during 1979 and 1999 across the New Zealand field on the Rothamsted Farm. The 2012/R/CS/726 field trial area was reserved for both *Phialophora* field trials 2015/R/WW/1516 and 2016/R/WW/1620. The field trial 2015/R/CS/760 was conducted by Vanessa McMillan and illustrates the position in the New Zealand field for comparisons of take-all infection with the 2015/R/WW/1516 field trial. NW = North West, E = East and SW = South West. P = plant available phosphorus (Olsen phosphorus) and K = exchangeable potassium. Not to scale.

Take-all infection was extremely low throughout the field trial, an average of 0.39% infected roots, and there does not appear to be a build-up of take-all inoculum. Although statistical differences were found across the varieties in their ability to build-up take-all inoculum, due to the low levels across the trial this data is unlikely to be biologically meaningful. No correlation was found between *Phialophora* colonisation and take-all infection of the elite wheat varieties, yet it is interesting that the elite wheat variety Alchemy had the lowest percentage of roots colonised with *Phialophora* and the highest percentage of roots infected with take-all. A first wheat and barley field trial (2015/R/CS/760) was also carried out in the field New Zealand by Vanessa McMillan (Figure 6.15). A higher average of 36% of roots infected with take-all was found in the soil core bioassay of the 2015/R/CS/760 field trial for the wheat plots (Vanessa McMillan, personal communication) compared to the 0.39% of take-all infected roots in 2015/R/WW/1516 field trial.

The above-ground phenotypic crop measurements of days to anthesis, yield and plant height can be seen to relate to corresponding agronomic features provided by the AHDB RL of 2013/2014 for the winter wheat varieties on ripening and yield. A strong positive relationship was found between the plant height of the field trial data and the plant height data provided by the AHDB RL of 2013/2014, yet only moderately positive relationships were found for anthesis and yield. This is due to the two sets of crop measurement data being taken across two different field seasons, with differences in environmental conditions, soil type and farm practice. A positive correlation was found between *Phialophora* colonisation when baited with Hereward and days to leaf senescence. This correlation is what you would expect to see with take-all in second and third wheats during moderate to high disease pressure, but not in a first wheat crop. This suggests that the later senescing elite wheat varieties might be selecting for *Phialophora* isolates which are particularly infective on Hereward. Alternatively, the higher levels of *Phialophora* colonisation could be causing premature root senescence.

A high yield was seen across the trial (17.83 - 25.57) and there appears to be no evidence of a detrimental effect of *Phialophora* colonisation on the yield of the plots. Wong (1975) conducted glasshouse experiments to look at the cross-

protection of *Phialophora* spp. against take-all disease and found that the grain yields of the plants inoculated with *Ggg* were higher than plants in soil without inoculated *Ggg*, yet not significantly different to the yield of healthy wheat plants. Wong et al. (1996) conducted field trials and found similar results; the grain yield of wheat inoculated with *Ggg* and *Phialophora* sp. lobed hyphopodia was not significantly different to the yield of healthy wheat in three out of four field experiments. The yield data in this chapter was particularly high due to the small size of the field plots (1m x 1m) which creates a large edge effect. In addition, the farm sprayed the entire field site with fertiliser, adding to the edge effect of the plots. This can be corrected for by adjusting the plot size to include half of the surrounding paths in the yield calculation; scaling down the yield values. However, it is not clear how much of this path area the plants would utilise to gain additional fertilisers, especially with the large path area of 3.5 m in the middle of the trial site (see Appendix 6.1). Additionally, it is not known if all varieties would scale by the same factor. Different varieties could differ in their ability to extract nutrients from the plot edges, which could be dependent on the root architecture or individual varieties. It was therefore decided that the yield data would not be recalculated, instead the data was ranked from the highest yielding variety through to the lowest yielding variety.

### 6.4.2 Pot bioassays

A pot bioassay method was developed to screen the ability of *Phialophora* spp. to colonise the roots of additional cereal genotypes. The calibration pot bioassays revealed discrepancies in the levels of *Phialophora* colonisation when visualising the roots by eye compared with under the light microscope. As expected, a greater level of colonisation could be seen with the aid of a microscope. Due to time constraints it was decided that root colonisation assessments would be visualised by eye for further pot bioassay experiments. The calibration pot bioassay of the sand maizemeal inoculum indicated that soil, rather than sand, provided the best growth medium for colonisation of both *Phialophora* species. This is in contrast to the take-all fungus which infects roots more successfully in sand. A ~50% level of colonisation of wheat seedling roots was found at 1:50 dilution of sand maizemeal

inoculum for the *Phialophora* spp. when assessing the roots visually by eye. A much lower level of root colonisation was found for *Phialophora* sp. lobed hyphopodia when compared to *Phialophora* sp., suggesting that this *Phialophora* species either does not grow as well in the sand maizemeal, that growth is slower than the *Phialophora* sp. or that the species is less pathogenic when colonising wheat. The aim to achieve ~50% roots infected was based on the take-all pot bioassay protocol. A 50% level of *Phialophora* colonisation should allow good discrimination between varieties in experimental pot bioassays.

A working pot bioassay protocol has now been devised from the AHDB summer bursary project in 2014 with undergraduate student Brittany Burton. The PDA plate inoculum pot bioassay provided a more consistent level of *Phialophora* colonisation and will be implemented in future work. Similarities to take-all were identified. The lowest level of *Phialophora* colonisation was seen in oats, which is completely resistant to take-all infection. Oats produce the antifungal saponin avenacin that can inhibit a range of fungal species, including *Ggt* and *Ggg* (Crombie et al., 1986, Osbourn, 1996). However, *Ggt* is known to grow epiphytically over the cortex of oat roots (Huber and McCaybuis, 1993). The presence of avenacin in the oat roots may have reduced the colonisation ability of the *Phialophora* species. Due to poor establishment of the winter oilseed rape in the reserved site of the 2016/R/WW/1620 field trial, spring oats were subsequently sown. It will be interesting to determine whether this has had an effect on the underlying populations of *Phialophora* spp. within the field site and whether the overall levels of *Phialophora* colonisation will differ from those in the 2015/R/WW/1516 field trial. Additional trials would be needed to test this and compare to the natural year to year variation that may be present due to other environment factors, for example rainfall and temperature. Oats are used as a break crop against take-all. Results suggested that the *Phialophora* sp. was a better coloniser of cereal roots than *Phialophora* sp. lobed hyphopodia. *Gaeumannomyces graminis* var. *graminis* (anamorph: *Phialophora* sp. lobed hyphopodia) has already been described as pathogenic on rice and Bermuda grass but weakly/non-pathogenic on cereals including wheat (Freeman and Ward, 2004). The species may be specifically

adapted to infect rice and less able to colonise other cereal genotypes. Extending the time of the pot bioassay may allow a higher level of colonisation by *Phialophora* sp. lobed hyphopodia. Rye indicated a low level of *Phialophora* sp. infection, suggesting that that rye could be activating a defence mechanism against the fungus as seen with take-all (Rothrock, 1988). The unnamed *Phialophora* sp. (isolate S.09.13) was not part of the distinct group in the phylogenetic tree of the additional isolates of the *Phialophora* isolate collection and the *Phialophora* spp. field isolates. The remaining genotypes appear to be equivalent in their level of *Phialophora* colonisation at the seedling stage. Triticale indicated a high level of colonisation for *Phialophora* in the pot bioassay when compared with pot bioassays screening take-all susceptibility where triticale is known to be moderately resistant to take-all infection (McMillan et al., 2014). The development of a working pot bioassay would allow the AHDB RL of winter wheat to be potentially screened in the future in order to compare findings with the field trial results.

### 6.4.3 Future work

The discussed field trial is currently being repeated in the reserved site in the New Zealand field (2016/R/WW/1620) (Figure 6.15 & Figure 6.16), due to the known presence of *Phialophora* populations in the soil, and will allow us to determine whether the results found across elite wheat varieties in 2014-2015 are consistent for a separate field season. It is important to repeat field trials over multiple years due to fluctuating environmental conditions. Take-all is known to be a patchy disease in the field and the ability of take-all inoculum to build-up in the soil is affected by a number of environmental conditions (Hornby et al., 1998, Gosme et al., 2007). It also is thought that *Phialophora* inoculum is fragmented throughout the soil (Wong, 1981). It is vital to interpret the 2014-2015 field trial results as trends until repeat field trial data is statistically analysed. In the future it would be necessary to screen the elite wheat varieties in different fields with different soil types and structure and different management practices as well as the presence of different isolates of *Phialophora* spp. to establish whether these factors affect the build-up of *Phialophora* populations in the surrounding soil of the wheat crop.



Figure 6.16. Aerial photograph of trial 2016/R/WW/1620 (within red box) in New Zealand field. (Photograph credit: Visual Communications Unit at Rothamsted Research).

The AHDB RL for winter wheat varieties may be producing different types or amounts of root exudates that could be supporting populations of *Phialophora* spp., and therefore resulting in different levels of *Phialophora* colonisation seen across the wheat varieties in this study. Fluorescent *Pseudomonas* spp., that produce the antibiotic 2,4-diacetylphloroglucinol (DAPG), are known to be a key component of the microbial community within the take-all decline (TAD) phenomenon (Raaijmakers and Weller, 1998). Kwak et al. (2010) found that *Phialophora* spp. isolates were less sensitive to 2,4-DAPG than *Ggt* isolates and the authors suggested that the *Phialophora* spp. isolates may be working with the 2,4-DAPG bacterial producers to help suppress *Ggt*. It would be interesting to conduct experiments to establish what the differences were in root exudates across the AHDB RL for winter wheat and whether these related to different microbial species associated with TAD as well as determining if the differences in root exudates were linked to the varying levels of *Phialophora* colonisation seen within this study.

During 2015, I submitted an application for a second AHDB summer bursary student but unfortunately I was unsuccessful. I devised a 10-week project to investigate the within season build-up of naturally occurring populations of *Phialophora* species for

selected varieties within the 2015/R/WW/1516 field trial. Monthly soil cores were to be taken from May (prior to the summer bursary student starting) through to August in order to track the build-up of *Phialophora* inoculum in the soil. Previous research has shown that take-all inoculum builds-up in the soil during a first wheat crop from May onwards (Slope and Gutteridge, 1979). *Phialophora graminicola* is known to build-up in the soil after two or more years of rye-grass (*Lolium* spp.) leys (Deacon, 1973b) and studies at Rothamsted Research have more recently confirmed this (Gutteridge et al., 2007). However, nothing is currently known about the ability of *Phialophora* species to build-up throughout the growth season under a cereal crop. This project would have provided novel information on the time course of the development of *Phialophora* in the field and how different wheat varieties influence this interaction.

Another unknown, not addressed within this PhD project, is how *Phialophora* species react to current fungicide applications. There are currently two commercially available fungicides that are effective as seed treatments for controlling take-all, fluquinconazole (Dawson and Bateman, 2001) and silthiofam (Schoeny and Lucas, 1999). Fluquinconazole is commercially marketed as Jockey® and is a quinazoline-based triazole that provides a broad spectrum of activity against a range of foliar and seed-borne diseases and Silthiofam is commercially available as the seed treatment Latitude®. Silthiofam is specific to take-all and is thought to target the mitochondrial adenine nucleotide transporter (Joseph-Horne et al., 2000) Foliar sprays of azoxystrobin, commercially available as Amistar®, and fluoxastrobin, commercially available as Fandango®, are also used as a control for take-all. If certain elite wheat varieties were found to be consistent in their ability to build-up *Phialophora* species in the soil, it would be vital to test how these fungicides affect *Phialophora* colonisation in the soil. This is an important consideration if *Phialophora* colonisation is to be used in the future as a successful biocontrol strategy.

### 6.4.4 Summary

In summary, this study has provided the first report of a subset of elite wheat varieties that have the ability to build-up natural populations of *Phialophora* fungi after a first wheat crop. This study only spans one field season and the repeat field trial is outside the timeframe of this PhD project. Initial findings suggest that there is potential for specific elite wheat varieties to be utilised to build-up natural populations of *Phialophora* in a first wheat and potentially provide an additional control strategy against take-all disease in a second wheat. Further work is required to understand the mechanistic process of *Phialophora* coloniation. More work is also needed on understanding the mechanism of *Phialophora* colonisation in the different elite wheat varieites and the ability of *Phialophora* to persist in the soil under field conditions. A working pot bioassay protocol has also been devised to allow cereal genotypes to be screened for *Phialophora* colonisation. Pot bioassay studies have found that similarities exist between *Phialophora* colonisation and take-all infection of cereal genotypes. The next step would be to use the pot bioassay to screen the ability of *Phialophora* fungi to colonise the seedling roots of the AHDB RL of winter wheat varieites, allowing the results to be compared with data obtained from screening adult plants in the field to natural populations of *Phialophora*.

# Chapter 7. General discussion

## 7.1 Project summary

The main aim of the PhD project was to characterise a range of diploid and hexaploid wheat germplasm that possess a promising level of take-all resistance under field conditions and to explore the genetic basis of resistance to take-all disease in the diploid wheat. In addition, the ability of natural populations of *Phialophora* species to colonise the roots of hexaploid wheat germplasm under field conditions was also explored. The main findings of the PhD project are discussed alongside the consideration of potential future research to be explored.

The most important finding within the take-all strand of the project was that third wheat field trials screening the diploid *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population revealed a good spread in susceptibility to take-all (Chapter 4). This provides the first known field screening of a *T. monococcum* mapping population to the susceptibility of take-all disease. Whilst the genetic basis of resistance has not currently been identified, a quantitative trait loci (QTL) analysis will be carried out within the next few months. This will provide an indication of the complexity of the resistance.

Other findings within the take-all strand of the project were less robust. Susceptibility to take-all was not consistent across two field seasons for the three moderately resistant hexaploid varieties screened in Chapter 3. The three hexaploid varieties only exhibited resistance during the low take-all disease pressure field season of 2014-2015. Differences were also found in the level of susceptibility of the *T. monococcum* accessions, during the 2013-2014 field season, between the field trial conducted in Chapter 3 and the trial conducted in Chapter 4, even though the two field trials were carried out in the same field and the grand mean of the take-all index (TAI) was almost identical across the two trials. Due to the inconsistency in susceptibility to take-all, it was also difficult to assess for differences in the soil water content between the three resistant and two susceptible varieties during the 2013-2014 field season. Added to this was the problem that there were plot variations in the measurement of the soil water

content. This made it difficult to compare the level of soil water content between plot replicates as well as across the five varieties and therefore the original hypothesis could not be answered. However, this is the first report of screening for differences in the soil water content between resistant and susceptible varieties to take-all disease in a third wheat situation. Initial results found that the soil water content increased with the level of take-all for three plot replicates of the highly susceptible variety Hereward and the moderately resistant variety Watkins 777 during the moderate disease pressure 2014-2015 field season. An increased level of plot replication and a spatial component to the analysis of the data, as required with the patchy nature of take-all, could help to further normalise the results.

The most important finding within the *Phialophora* strand of the project was the results in Chapter 6 that resulted in the first indication that elite wheat varieties on the AHDB Recommended List (RL) differ in their potential to build-up *Phialophora* spp. under a first wheat crop. Although the findings are only represented by one field season and therefore care must be taken when interpreting the results, the current findings are highly promising and a repeat field trial will be assessed to provide a comparison across a separate field season. There is the potential that a farmer could choose a first wheat variety from the AHDB RL of winter wheat that could build-up *Phialophora* populations under the crop, providing a level of protection against take-all disease in a second wheat crop. *Phialophora* spp. are not the only fungi within the *Gaeumannomyces-Phialophora* complex that can be built-up under a wheat crop (McMillan et al., 2011). The finding complements another study that has identified that there are differences in the ability of wheat varieties to build-up take-all inoculum under a first wheat crop, named the take-all inoculum build-up trait (TAB) (McMillan et al., 2011). A sub-set of the AHDB RL trials have recently been sampled by Vanessa McMillan to explore whether the TAB trait functions within the elite varieties and there appears to be differences across the RL in their ability to build-up take-all inoculum (Vanessa McMillan, personal communication).

*Phialophora* spp. are known to build-up under grass leys (Deacon, 1973a, Gutteridge et al., 2007) and evidence has suggested that populations of

*Phialophora* sp. lobed hyphopodia (Wong and Southwell, 1980) and *Phialophora graminicola* decline under cereal crops (Slope et al., 1978). However, *Phialophora* populations have persisted on the Rothamsted Farm without the use of grass leys. This coupled with the results from the field trial results in Chapter 6, suggest that the dynamics of the soil microbial community under differing plant species is more complex than initially thought. Mauchline et al. (2015) studied the abundance of *Pseudomonas* spp. as part of the rhizosphere microbial community under both high and low TAB wheat varieties and *Pseudomonas* populations were affected by the variety chosen as a first wheat crop. Mauchline et al. (2015) found that following the high TAB wheat variety Hereward, there was an increase in the abundance of *Pseudomonas fluorescens*, a species that is antagonistic to other microbes. Mehrabi et al. (2016) has recently found that a decreased biodiversity of *Pseudomonas* spp., is correlated with a reduced level of take-all severity in second wheat crops. This contradicts previous thinking that a high biodiversity is linked with an increase in disease suppression. These studies highlight the complex nature of the population structures of soil microorganisms under selected cereal monocultures.

Arguably as important, was the development of a draft genome for three *Phialophora* species in Chapter 5. A UK *Phialophora* isolate collection was gathered (additionally utilised in Chapter 6) and from this an isolate representing each of the three *Phialophora* spp. was sequenced and the genomes were assembled and annotated. Although there were differences in the coverage and the extent of assembly across the three genomes, these are the first *Phialophora* reported draft genomes and they provide the first glimpse into the similarities that *Phialophora* spp. have with the closely related take-all fungus. The genomes will also provide a valuable tool for inter-genome comparisons of species within the *Gaeumannomyces-Phialophora* complex and their differences in colonisation/infection process.

A working pot bioassay protocol was developed in order to screen additional cereal varieties to *Phialophora* species, using the isolate collection gathered in Chapter 5. Although the pot bioassay revealed similarities between *Phialophora* colonisation and take-all infection of cereal varieties, rye indicated a significantly lower level of

colonisation of the unnamed *Phialophora* sp. during the seedling stage. Rye is known to be highly resistant to take-all infection (Rothrock, 1988) and rye could also be exhibiting a level of resistance to the unnamed *Phialophora* sp. The taxonomic status of this *Phialophora* sp. is currently unresolved (Hernández-Restrepo et al., 2016).

### 7.2 Further analyses

Further research at Rothamsted will focus on screening the susceptibility of a hexaploid mapping population from varieties screened during this PhD project as well as screening an additional *T. monococcum* mapping population to take-all disease. Further analyses are also planned to assess the repeat field trial screening the ability of the AHDB RL of winter wheat to build-up natural populations of *Phialophora* fungi. Furthermore, this section details research that could potentially be carried out in the future to accelerate our knowledge on both the pathogenic take-all fungus and the antagonistic *Phialophora* fungi.

A mapping population has been developed and 94 lines are currently being screened in a third wheat situation in the field between the moderately resistant Watkins variety 777 and the susceptible variety Paragon, which were screened individually in the field in Chapter 3. The spring wheat variety Paragon has been used as a UK reference genotype within the Wheat Genetic Improvement Network (WGIN) programme. During the PhD field trial season of 2013-2014, detailed in Chapter 3, the moderately resistant Watkins variety 777 and the susceptible variety Paragon could not be discriminated from one another on their susceptibility to take-all, whereas in other field trials at Rothamsted, parental differences were still evident (Vanessa McMillan, personal communication). Resistance to take-all is less likely to be expressed under very high disease pressures (Scott, 1981), and it can also be difficult to discriminate between take-all resistant and susceptible varieties under low disease pressure. Yet the 2013-2014 field season demonstrated a moderate take-all disease pressure. This highlights that the moderate resistance found in Watkins line 777 may not be consistent across different environmental conditions and disease pressures and there may be difficulties in identifying the regions or genes responsible for the partial resistance in the Watkins line 777 from

the screening of the mapping population. However, analysis of multiple years of field trial data on this population, combined with careful selection of previously discriminatory field sites, could potentially be used to identify QTLs that are stable across the year. This approach may provide immediately useable resources for future wheat breeding programmes, as well as identity less desirable QTLs that are associated with individual seasons and environmental conditions. Also, this hexaploid population could identify the best lines from the cross to take forward for further mapping population development. In addition, eight further Watkins X Paragon mapping populations through the single seed descent (SSD) system are currently under development at the John Innes Centre (JIC) as part of the BBSRC funded Wheat Improvement Strategic Programme (WISP) for future field phenotyping at RRes. This will allow further comparison between populations.

As discussed in Chapter 4, the genotyping data of the *T. monococcum* MDR037 (S) X MDR046 (R) mapping population was returned from Bristol at the end of August 2016. This data will be analysed and a QTL analysis will be carried out over the subsequent months, allowing the first analysis of the complexity of the genetic basis of resistance to take-all disease in *T. monococcum* and providing an indication of whether there is the potential of introgressing the resistance trait into hexaploid wheat. The first crosses/back crosses between resistant *T. monococcum* genotypes and hexpaloid wheat are underway in the WGIN project. If major QTLs are found, it does not necessarily mean that the introgression into hexaploid wheat will be successful. There is the potential that once the take-all resistance is introgressed into hexaploid wheat, there could be suppression of the resistance, as has been observed when attempting to control other pathogens via the introgression of traits from ancestral species (Jin et al., 2007, Saintenac et al., 2013). It is known that polyploidy improves the expression of a number of traits, yet it can often come at the expense of supressing the expression of resistance from negative interactions of loci between genomes (Wulff and Moscou, 2014). The field screening of the *T. monococcum* mapping population is currently being repeated for a third year to validate and confirm any identified QTLs.

The *T. monococcum* mapping population developed between the partially take-all resistant accession MDR031 and the take-all susceptible accession MDR043 is now at the F6 stage and 120 lines will be screened in a third wheat situation at RRes in the 2016-2017 field season. The *T. monococcum* accession MDR031 has previously demonstrated a slightly higher level of resistance to take-all as well as MDR043 exhibiting a higher susceptibility to take-all (McMillan et al., 2011), illustrating a larger amount of phenotypic variation amongst the parental lines and potentially a higher probability of recombination.

As described in Chapter 5, the *P. graminicola* species could not be isolated from the Rothamsted Farm. This was unexpected since *P. graminicola* has previously been documented as the most common soil-dwelling *Phialophora* spp. in the UK and *P. graminicola* has previously been isolated from the Rothamsted Farm (Ward and Gray, 1992). This suggests that a population shift may have occurred across the Rothamsted Farm. *Phialophora graminicola* is known to build-up in the soil after two or more years under grass leys (Deacon, 1973b, Gutteridge et al., 2007). *Phialophora* populations have persisted in fields across the Rothamsted Farm without the inclusion of grass leys in the crop rotation. It would be interesting to sample from regions inside, as well as outside, of the UK that continue to utilise grass leys or from regions in permanent grassland to establish the *Phialophora* spp. population structure and to compare this to the population structure found across different arable sites, including the Rothamsted Farm. It would also be worth attempting to establish the effect of different arable management practices, such as direct drilling and tilling, on the population levels of *Phialophora* fungi in the soil.

The newly sequenced *Phialophora* draft genomes provide a preliminary tool to allow comparisons between the genomes and predicted proteome of species within the *Magnaporthe-Gaeumannomyces-Phialophora* complex. Species within this complex are able to colonise a wide range of plant hosts and tissues. Recently, Luo et al. (2015) conducted a phylogenomic analysis to understand the infection modes of species within the Magnaporthales order. Three clades were identified: one clade comprised of saprotrophs associated with submerged wood, a second clade included the pathogens that cause blast disease of monocots and the third clade

contained species that penetrate root tissue with hyphopodia, such as the take-all fungus and *Phialophora* spp. (Luo et al., 2015). The phylogeny was based upon a set of conserved single-copy gene markers, similar to the Benchmarking Universal Single-Copy Orthologs (BUSCO) analysis carried out in Chapter 5, yet the study by Luo et al. (2015) did not include *Phialophora* sp. lobed hyphopodia and the unnamed *Phialophora* sp.

Future work could focus on providing transcriptome sequencing data of the three *Phialophora* spp. along with *Ggt,* allowing a comparison in the expression levels throughout the different stages of root colonisation by *Phialophora* fungi and root infection by the take-all fungus. This could help to provide vital knowledge of the different modes of infection versus colonisation of the two closely related fungi. A recent study by Yang et al. (2015), has provided the first comparison of the whole transcriptome of *Ggt* growing in culture and *Ggt* infecting wheat roots. Almost twice as many differentially expressed genes (65%) were upregulated than downregulated (35%), providing a large amount of candidate genes for future research on virulence mechanisms of *Ggt*. However, a reliable transformation system for *Ggt* is still lacking, hindering future gene disruption analyses.

The repeat field trial in Chapter 6, screening the ability of the AHDB RL of winter wheat to build-up natural populations of *Phialophora* spp., will be assessed to provide two years of field trial data. If the repeat field trial confirms the results from the 2014-2015 field season, further work would be needed to explore whether the build-up of *Phialophora* fungi occurs externally to the field on the Rothamsted Farm. As briefly discussed in Chapter 6, the elite wheat varieties would need to be screened in different fields across the UK to take into account different soil types and soil structures on both the *Phialophora* species type and population levels of the fungi and how this affects the varietal build-up of the *Phialophora* spp. McMillan et al. (2011) previously demonstrated that there are differences in the ability of wheat varieties to build-up take-all inoculum in the soil on the Rothamsted Farm when grown as a first wheat crop and the AHDB RL trials have recently been sampled, by Vanessa McMillan, to explore whether the TAB trait functions within the elite varieties of the RL and across different regions and

therefore varying soil types and take-all population structures. In addition, the *T. monococcum* accessions are currently being screened in the field at RRes to determine whether the accessions vary in their ability to build-up take-all inoculum. The AHDB RL trials could be sampled to explore whether the build-up of *Phialophora* species, under a first wheat crop, can be demonstrated across different UK sites, as well as screening elite wheat varieties from the most recent RL list. McMillan et al. (2011) demonstrated that when a low take-all inoculum builder was grown as a first wheat, the subsequent second wheat had less take-all disease and an increase in yield. It would be necessary to perform field trials to assess the effect that the build-up of *Phialophora* fungi, under a first wheat crop, had on the level of take-all disease and grain yield in a second wheat crop by oversowing the first wheat plots with a second wheat variety. Once the repeat trial during the 2015-2016 field season is assessed, it will be worth examining whether there is an overlap of wheat varieties that represent a low TAB and a high builder of *Phialophora* fungi.

An additional study could utilise a metagenomics analyses to provide an insight into the soil microbial composition associated with *Phialophora* spp. as well as the differences in the microbial community under an elite wheat that has shown the capacity to build-up *Phialophora* species compared to a variety that cannot build-up natural populations of *Phialophora*. The endophytic fungus *Piriformospora indica* has been found to colonise wheat roots and can inhibit the growth of *Ggt* (Ghahfarokhy et al., 2011). Rabiey et al. (2015) has also shown that the endophyte can protect wheat seedlings from Fusarium disease in glasshouse conditions. It is unknown how *P. indica* interacts with other soil microorganisms, yet the fungus is known to persist in simulated UK autumn conditions (Rabiey et al., 2015). It would be interesting to look at the effects that the endophyte has on *Phialophora* spp., *P. indica* are known to inhibit *Ggt* with the development of inhibition zones in culture (Ghahfarokhi and Goltapeh, 2010), and whether you could combine the two species to look at the suppression on take-all.

### 7.2.1 Young Entrepreneur Programme project application

During my 4th year of my PhD project, I applied to the Young Entrepreneur Programme (YEP) at Rothamsted Research, a funding programme aimed at final year PhD students. The YEP scheme requires applicants to pitch a project idea that is innovative and would deliver a novel solution to a challenge that is relevant to the Rothamsted mission. I proposed a project to develop a diagnostic loop-mediated isothermal amplification (LAMP) assay to allow farmers to detect *Phialophora* spp. in their soil and to therefore aid in the farmers'/farm advisors' decision masking as to whether to grow a second wheat crop. The proposed project would have followed on from my PhD project and it would have allowed further analysis of the *Phialophora* draft genomes in order to develop *Phialophora* species-specific primers for a diagnostic assay. Unfortunately, I was unsuccessful in securing the funding, but I am still exploring ways to obtain future funding.

The main objective of the proposed project was to develop and validate a LAMP diagnostic method to detect *Phialophora* fungi from the soil by performing further work on the sequenced *Phialophora* draft genomes, in Chapter 5, in order to develop species-specific primers that would discriminate the *Phialophora* species from the take-all fungus as well as other soil-dwelling microorganisms. The simplicity of the LAMP assay protocol makes it ideal for in-field use. The second objective was to test methods of extracting *Phialophora* DNA from the soil and to establish a soil sampling protocol. A soil sampling protocol would have to be devised to establish how many soil sampling points and how much soil would need to be provided by the farmer in order to detect the presence and to quantify *Phialophora* fungi in the soil. *Phialophora* inoculum is known to be patchy throughout the soil (Wong and Southwell, 1980), as seen with take-all (Hornby et al., 1998), and therefore a sampling protocol would need to be devised to sample as much of the area as possible.

The proposed project had opportunities for future progression. Firstly, a database could have been established to store information gathered from the sampled farmer's fields. Information on the *Phialophora* species and *Ggt* distribution along with soil types, cropping histories and meteorological data from sampled fields

could all be included. The farmer would then be able to access the database, along with the data being a potential tool for scientific researchers and industry. Discussions would have been carried out during the year to establish initial links for the set-up of the database, it may have been possible to include this information with the current CROPROTECT App (https://croprotect.com/), since Toby Bruce at Rothamsted Research is leading the research. CROPROTECT is a web based knowledge exchange tool where farmers and agronomists can access information on integrated pest management (IPM) (https://croprotect.com/). Information and management recommendations for take-all disease have been provided on the CROPROTECT web site by Vanessa McMillan. There could also be the opportunity to provide distribution maps on the prevalence of *Phialophora* fungal species across the UK. The PreDicta B® (B = broadacre) DNA-based soil testing service for soil-borne pathogen identification, currently provides maps on the distribution and potential risk level of both *Ggt* and *Gaeumannomyces graminis* var. *avenae* (*Gga*) across the cropping regions of Southern Australia, provided by the South Australian Research and Development Institute (SARDI) (Ophel-Keller et al., 2008). (http://pir.sa.gov.au/research/services/molecular_diagnostics/predicta_b).

### 7.3 DTP laboratory rotation

As part of the DTP at The University of Nottingham, three 6 week laboratory rotations were carried out. A brief description of the 6 week laboratory rotation carried out under this PhD project is detailed below.

One of the original objectives of the PhD project was to characterise the cellular aspects of the take-all root resistant phenotype in seedling pot bioassays with the related rice blast fungus *Magnaporthe oryzae*. Rice blast is traditionally a foliar disease caused by the fungus *M. oryzae* (previously named *M. grisea*) that infects rice plants and is closely related to *Ggt* (Dufresne and Osbourn, 2001). *Magnaporthe oryzae* is considered a model phytopathogen due to the ability to culture the fungus in laboratory conditions and in-depth knowledge in the literature of the molecular interactions between the fungus and its host (Valent and Chumley, 1991, Talbot, 2003). *Magnaporthe oryzae* can infect a number of host plants though colonisation of the leaf tissue (Talbot, 2003) but Dufresne and Osbourn (2001) have

also demonstrated that *M. oryzae* can infect and subsequently cause disease in cereal roots. A lack of knowledge exists about what factors are necessary for colonisation of roots by soil-borne fungal pathogens (Dufresne and Osbourn, 2001) and established genetic tools may allow us to utilise *M. orzyae* to expand our knowledge on *Ggt* infection. A 6 week project was carried out during the laboratory rotations in the first year of the PhD project, aimed at replicating the results of Dufresne and Osbourn (2001) in demonstrating that *M. oryzae* can infect cereal roots, a potential surrogate species for *Ggt* infection, and to determine whether genotypes that are resistant or susceptible to *Ggt* infection are also resistant or susceptible to *M. oryzae* infection.

During the 6 week laboratory rotation, a 4 week pot bioassay was carried to inoculate the parental lines of the *T. monococcum* mapping population that were screened in Chapter 4, the variety Hereward as the take-all highly susceptible control and oats as the take-all resistant control species with a Green Fluorescent Protein (GFP)-tagged *M. oryzae* isolate and the take-all fungus. The inoculated seedlings were visually assessed for *M. oryzae* and *Ggt* infection as well as carrying out PCR assays and qPCR assays to quantify the amount of fungal biomass. Results showed that visual assessment and qPCR data for the take-all pot tests were not always consistent. Additionally, there were no differences in the susceptibility of the two *T. monococcum* accessions to take-all infection in the pot bioassay. Therefore, the pot bioassay results do not replicate results previously found in the field (McMillan et al., 2014), additionally shown in Chapter 4. There was also no difference in the susceptibility of the *T. monococcum* accessions to *M. oryzae* infection. The objective was not continued due to more promising leads and time constraints arising from the *Phialophora*-based project objectives.

A query was raised by the Health and Safety Executive (HSE) in January 2011 as to whether the GFP-tagged *M. oryzae* could spread systemically from the root tissue to aerial tissue. A report was sent with the results of the above pot bioassay to confirm that *M. oryzae* infection was not visibly transferred to the aerial parts of the plants and no airborne spores were formed.

## 7.4 Recent changes to the nomenclature of species within the *Gaeumannomyces-Phialophora* complex

Gams (2000) proposed a new genus, *Harpophora*, for anamorphs within the *Gaeumannomyces-Phialophora* complex (Table 7.1). There has been varied acceptance of the new genus name, with a number of scientific papers adopting the new proposed *Harpophora* name (Yuan et al., 2010, Su et al., 2013), whilst many have kept the *Phialophora* nomenclature (Freeman and Ward, 2004, Gutteridge et al., 2007, Kwak et al., 2010). This has led to further confusion within the literature, from an already disordered nomenclature system, since *Phialophora* is an extremely polymorphic genus that contains anamorphs from *Discomycetes*, *Pyrenomycetes* and *Loculoascomycetes* (Gams, 2000). The proposed *Harpophora* genus contains four new species combinations: the type species *Harpophora radicicola* (*Phialophora radicicola* (Cain, 1952)), *Harpophora maydis* (*Cephalosporium maydis* (Samra et al., 1963)), *Harpophora graminicola* (*Phialophora graminicola* (Walker, 1980)) and *Harpophora zeicola* (*Phialophora zeicola* (Deacon and Scott, 1983)). These names comprise solely of the anamorphs within the *Gaeumannomyces-Phialophora* complex without the need of separate names (Table 7.1).

The *Phialophora* nomenclature, for anamorphs within the *Gaeumannomyces-Phialophora* complex, was chosen for use throughout this PhD project due to the lack of complete uptake of the proposed genus name of *Harpophora* in the current literature (Table 7.1). However, a recent study by Hernández-Restrepo et al. (2016) has reassessed the phylogeny of species and varieties within *Gaeumannomyces* and changes to species studied within this PhD project are detailed in Table 7.1. The study has revealed that *Ggt* and *Gga* cluster outside of the *G. graminis* clade and are now considered to represent different species, *G. tritici* and *G. avenae* respectively. *Phialophora radicicola, P. zeicola* and *Gaeumannomyces graminis var. maydis* (*Ggm*) (teleomorph of *P. radicicola* and *P. zeicola*) have previously been confirmed as identical from ITS sequence analysis (Ward and Bateman, 1999) and Hernández-Restrepo et al. (2016) found that the species fell within the Radicicola clade of *Gaeumannomyces*, using the name of *G. radicicola* (Luo et al., 2015) to

encompass the two species with *Ggm* a synonym of *G. radicicola*. *Phialophora* sp. lobed hyphopodia isolates also fell within the Radicicola clade of *Gaeumannomyces* and a new species name of *Gaeumannomyces hyphopoides* was proposed. The *Slopeiomyces* genus within *Magnaporthaceae* was previously proposed by Klaubauf et al. (2014) to accommodate *P. graminicola* (*G. cylindrosporus*) and the species name *S. cylindrosporus* has been kept. The unnamed *Phialophora* sp. GP57 isolate, previously isolated at RRes (Ward and Bateman, 1999) and discussed in Chapter 6, was found to represent a distinct lineage within *Magnaporthaceae*, yet the isolate was found to be sterile so a formal species name is currently unavailable (Hernández-Restrepo et al., 2016).

Table 7.1. Nomenclature of species within the *Gaeumannomyces-Phialophora* complex that are referred to in this PhD project and recent updates.

| Original name | Current name used throughout PhD project | Previously proposed | Most recent nomenclature |
|---|---|---|---|
| *Ophiobolus graminis* var. *avenae*[1] | ***Gaeumannomyces graminis* var. *avenae***[2] | | *Gaeumannomyces avenae*[3] |
| *Ophibolus graminis*[4] | ***Gaeumannomyces graminis* var. *graminis***[5,6] | | *Gaeumannomyces graminis*[3] |
| *Ophibolus graminis*[4] | ***Gaeumannomyces graminis* var. *tritici***[5,6] | | *Gaeumannomyces tritici*[3] |
| | ***Phialophora sp.***[7,8] | | *Magnaporthaceae, incertae sedis*[3] |
| | ***Gaeumannomyces graminis* var. *graminis* (teleomorph)**[6] | | *Gaeumannomyces hyphopoides*[3] |
| | ***Phialophora sp. lobed hyphopodia* (anamorph)**[9] | | *Gaeumannomyces hyphopoides*[3] |
| | ***Gaeumannomyces cylindrosporus* (teleomorph)**[10] | | *Slopeiomyces cylindrosporus*[11] |
| *Phialophora radicicola* var. *graminicola*[9] | ***Phialophora graminicola* (anamorph)**[12] | *Harpophora graminicola*[13] | *Slopeiomyces cylindrosporus*[11] |
| | ***Gaeumannomyces graminis* var. *maydis***[14] | | *Gaeumannomyces radicicola* (syn.)[3] |
| *Phialophora radicicola*[15] (var. *radicicola*[9]*)* | ***Phialophora radicicola***[9] | *Harpophora radicicola*[13] | *Gaeumannomyces radicicola*[3] |
| | ***Phialophora zeicola***[16] | *Harpophora zeicola*[13] | *Gaeumannomyces radicicola*[3] |
| *Magnaporthe grisea*[17] | ***M. grisea/Magnaporthe oryzae***[18] | | *Magnaporthe oryzae*[18] |

[1] Turner (1940). [2] Dennis (1960). [3] Hernández-Restrepo et al. (2016).

[4] Prillieux and Delacroix (1890). [5] Arx and Olivier (1952). [6] Walker (1972).

[7] Ward and Bateman (1999). [8] Ulrich et al. (2000).

[9] Deacon (1974). [10] Hornby et al. (1977). [11] Klaubauf et al. (2014). [12] Walker (1980). [13] Gams (2000).

[14] Yao et al. (1992). [15] Cain (1952). [16] Deacon and Scott (1983).

[17] (Barr, 1977). [18] Couch and Kohn (2002).

## 7.5 Utilisation of resistance genes and QTLs in wheat breeding programmes

Wheat breeding programmes aim to improve the yield potential of varieties, whilst improving the crop quality and providing desirable traits such as resistance to pests and pathogens. Providing pest and disease control through plant genetics, rather than the application of chemistry, is a far more desirable approach for farmers. As discussed and utilised in Chapter 3 and Chapter 4, industry programmes such the WGIN and the WISP consortium have provided a toolkit of pre-breeding material to be screened and to potentially exploit useful traits for future wheat breeding programmes. The two industry programmes are essential collaborations between publicly funded scientific institutes and universities with private breeding companies, allowing the direct delivery of wheat improvement traits into current wheat breeding programmes.

Quantitative trait loci (QTLs) have been identified and the development of markers, through marker assisted selection (MAS), to select for individual plants with a desired trait, such as drought tolerance in pea breeding programmes (Iglesias-García et al., 2015) and Fusarium ear blight (FEB) resistance in wheat breeding programmes (Pumphrey et al., 2007). The bread wheat (*Triticum aestivum*) genome has recently been sequenced (Mayer et al., 2014), providing a substantial amount of sequencing data that could only accelerate the mapping of QTLs in MAS and could provide an increase in the utilisation within plant breeding programmes. The main advantages of MAS in plant breeding programmes were detailed in Chapter 4. However, there has been a lack of exploitation of QTLs in plant breeding programmes (Bernardo, 2008), and recent advances in genomic selection have gathered expectations to allow markers to be used in breeding programmes for complex traits (Heslot et al., 2015). Disease resistance is often known to be highly complex and identified QTLs for traits can often have inconsistent effects from differing environmental conditions, as discussed in Chapter 4. There is also the need to combine resistance for multiple disease, rather than just a single disease (as evaluated within this PhD project) and to assess for trade-offs between disease resistance and other beneficial traits (Summers and Brown, 2013).

Genomic selection is an alternative to MAS that estimates genetic marker effects covering the whole genome of the population based upon the prediction model of a training population that consists of both phenotypic and genetic information (Meuwissen et al., 2001). Genomic selection has previously been utilised in the animal breeding industry (Lund et al., 2011). Genomic selection can therefore be used by breeders to statistically model how a plant will perform and can allow interactions such as genotype x environment to be incorporated, predicting performance in different environments (Desta and Ortiz, 2014). Massman et al. (2013) provided the first evidence that genomic selection can work in crops and the authors found that there were gains from selection that were greater when using all available genetic markers over a subset of markers from MAS. There is a huge potential for genomic selection to be used for crop improvement but it is currently unclear how feasible this will be. INRA in France has been trialling genomic selection in their winter wheat breeding programme and initial comparisons with field results are encouraging (Gilles Charmet, personal communication at Wheat Genetics Course, 2015). Although phenotyping data is not directly used for selection, phenotyping data is still relied upon during the development of the training population. The process of phenotyping populations will continue to be an important role in the process of wheat breeding and there is the need for advances in high-throughput whole-plant phenotyping methods (Dhondt et al., 2013); in turn these advances will improve the efficiency of genetic selection (Desta and Ortiz, 2014). It will be interesting to see how the genomic selection technology develops and whether it is adopted by other wheat breeding institutes in the future or whether MAS will continue to be a more reliable procedure. The two methodologies could also be used collectively, with MAS feeding into the genomic selection models (Kuijken et al., 2015). The utilisation of plant host resistance in elite wheat varieties is considered the most environmentally friendly method to control against wheat diseases and further advances into predictive models could only accelerate the potential deployment of resistance genes for soil-borne diseases into current varieties. Improvements in varietal resistance to wheat pathogens is vital, especially with the need to minimise chemical applications, as discussed in Chapter 1. Genomic section could allow lines to be selected that are

carrying specific markers throughout the genome that are associated with complex traits such as take-all resistance, as demonstrated in Chapter 4, and yield, whilst also allowing the selection of a gene for novel trait (Summers and Brown, 2013).

### 7.6 Future root phenotyping research and technologies

Recently, there has been an increased realisation of the importance of optimising root traits to help increase the yield potential of crops, a previously underexplored area that has significant prospects. This has resulted in an acceleration in the development of crop root phenotyping technologies. Scientists and plant breeders have been faced with the challenge of overcoming the limiting factor of gaining high-throughput phenotypic data of root traits in the face of recent advancements of high-throughput genotyping of traits (Kuijken et al., 2015). There has often been an assumption by plant breeders that selection for yield will indirectly lead to a selection of optimal root systems (Wasson et al., 2012), however, directly selecting for specific root traits can in turn increase the yield potential of the crop and/or improve root performance under stressful conditions such as drought, salinity and the presence of soil-borne diseases.

Gaining phenotyping data for foliar disease resistance is fairly straightforward compared to gaining phenotyping data on root traits such as soil-borne diseases, as demonstrated within this PhD project. There has also been the need for progress in the development of phenotyping technologies for root architecture traits; Chapter 3 described and demonstrated some of the difficulties in obtaining root architecture measurements from field experiments. Recent advances in laboratory based technologies have included 2D imaging platforms that allow 3D root reconstructions and quantification of root traits (Topp et al., 2013, Pineros et al., 2016) along with a vast range of supporting imaging software (see Lobet et al. (2013) for an online database of over 90 image data processing software tools), yet these imaging systems can only scan small pot experiments. A transparent soil has been developed by Downie et al. (2012), allowing the utilisation of 3D imaging to study the mechanism of the transmission of *Escherichia coli* onto lettuce; a public health hazard. The transparent soil can be used to grow a whole range of host plants *in situ* and this technology will allow root-microbe interactions to be further

explored. Downie et al. (2014) have also used the technology to image the distribution of plant growth promoting rhizobacteria along the surface of lettuce roots. This technology could be exploited in the future to image the runner hyphae and hyphopodia in the initial stages of take-all infection/*Phialophora* colonisation in a pot bioassay.

Wasson et al. (2016) have demonstrated automated in-field phenotyping platforms with the development of a portable fluorescence imaging system allowing root measurements to be taken from soil cores. Large scale in-field root phenotyping advances include complexes such as the rhizo-lysimetry system in Australia, allowing direct root observation in root growth and soil water content (Eberbach et al., 2013). Chapter 3 demonstrated the difficulties in obtaining soil water content measurements in relation to take-all susceptibility, due to plot variation between soil moisture sensors from soil type and structural differences. The rhizo-lysimetry system (Eberbach et al., 2013), along with a number of experimental field studies (Whalley et al., 2008), use soil moisture sensors and access tubes to measure volumetric soil water content through the soil profile and this technology could be used in the future to monitor changes in soil water content at different depths within field experiments screening the susceptibility of wheat varieties to take-all disease. Long-term simulation models by Lilley and Kirkegaard (2016) have demonstrated that overestimations have previously been made on the importance of deep rooting varieties in capturing water in shallow soils in Australia, highlighting the potential of utilising crop modelling to identify root traits that may incur a yield loss rather than gain.

In-field plant phenotyping platforms (as demonstrated by the LemnaTec Field Scanalyzer at RRes) (http://www.bbsrc.ac.uk/news/food-security/2015/150713-pr-worlds-first-field-scanalyzer-at-rothamsted-research/) are currently ahead of the measurement abilities of in-field root phenotyping platforms. Although take-all is a root disease, in-field plant phenotyping platforms and unmanned aerial vehicles (UAV) (as demonstrated by the UAV Octocopter technology being used at RRes) have the potential to capture images of the characteristic take-all patches and the above-ground symptoms, described in Chapter 1, during severe take-all epidemics.

The use of sensors and imaging on such plant phenotyping technologies also have the potential of detecting and measuring foliar plant pathogens, for example infrared thermography can measure the temperature changes in response to pathogen infections and fluorescence imaging can measure differences in the photosynthetic ability of the infected plant. Potentially, thermal imagery could be tested for its ability to provide an indirect quantitative measurement of root health and function in the field.

### 7.7 Future of take-all control

The future of take-all control relies upon an integrated management strategy due to the complex nature of the environmental factors that affect the severity of take-all disease. Two of the main themes explored within this PhD project have focused upon two differing control strategies against take-all disease, identifying genetic resistance to take-all disease in the diploid *T. monococcum* wheat (Chapter 4) as well as exploring the potential of building up natural populations of *Phialophora* spp. under a first wheat as a potential suppressant of take-all in a second wheat (Chapter 6).

Host plant resistance to *Ggt* is not currently utilised as a form of resistance to take-all, yet it is considered as the most favourable form of control against plant pathogens due to social, economic and environmental reasons (Mundt, 2014). However, one of the main challenges of releasing new resistance genes against fungal pathogens into the environment, is the durability of the resistance. Durable resistance has been defined as host plant resistance that continues to be an effective control measure whilst the wheat variety is widely cultivated (Johnson, 1981) and it is one of the main aims for wheat breeders to attain in breeding strategies. Combining a number of resistance genes or quantitative resistance loci (QRL) can improve the durability of plant host resistance. The genetic basis of take-all resistance is currently being explored (Chapter 4), whilst other sources of resistance are also being investigated within the take-all research group at RRes and could lead to the potential of stacking resistance genes and or QTLs. However, the durability of resistance is not due entirely to the genetics of the host plant resistance, other factors come in to play such as the population structure and

genetics of the plant pathogen (McDonald and Linde, 2002). Genetic diversity within fungal populations can affect the durability of host plant resistance (Irzykowska, 2012). As described in Chapter 1, there are two main genetic groups of *Ggt* isolates that have been found to coexist within the same fields (Daval et al., 2010). *Gaeumannomyces graminis* var. *tritici* is homothallic (Hornby et al., 1998) and although laboratory studies have indicated that sexual recombination can occur between genetically different *Ggt* isolates (Pilgeram and Henson, 1992), it is presumed that there is either no or very rare events in the genetic exchange between the two main genetic groups of *Ggt* in the field (Hornby et al., 1998, Lebreton et al., 2004). This coupled with a low population size of the *Ggt*, when the host plant resistance is released, could lead to an increase in the durability of the resistance. Pathogens with a low population size have less of an evolutionary potential than pathogens with a high population size and are therefore less likely to overcome host plant genetic resistance (McDonald and Linde, 2002).

Studying the potential of elite wheat varieties to build-up natural populations of *Phialophora* spp., during this PhD project (Chapter 6), could act as a possible management strategy to help to reduce the population size of *Ggt* isolates in farmer's fields. This could possibly lead to the utilisation of two control strategies in the future to help combat take-all disease by reducing the population size of *Ggt* isolates and the release of the first plant host resistance. The development of such future control strategies for plant pathogens is vital since the development of new fungicide chemistry is currently slow due to the development of fungicide resistance, it is difficult to develop fungicides against soil-borne diseases and the restricting policies and bans of certain fungicides under the introduction of EU pesticide legislation Directive 2009/128/EC, which has led to fewer chemicals being approved.

### 7.8 Future of root diseases in wheat crops in a global context

There is a growing need for crop modelling to estimate the projected climate change effects and subsequent effects of wheat diseases on the yield potential of wheat crops. Ultimately, these models help to provide information for government and inter-government strategies as well as new and optimal wheat breeding

strategies within global industry. A number of crop models have estimated that under differing climate change scenarios, wheat yield potential will decrease (Semenov et al., 2014, Asseng et al., 2015). Semenov et al. (2014) used the SIRIUS wheat model which provided a prediction of an increase in the heat stress on the plant at both the meiosis and anthesis time point (Semenov et al., 2014). Currently, there appears to be an unknown speculative effect of climate change on the response of *Ggt* across the UK (West et al., 2012). Yet, the projected increase in heat stress during anthesis (Semenov et al., 2014) could help to exacerbate the severity of take-all disease symptoms (as discussed in Chapter 1) in Europe. Boland et al. (2004) have estimated that the effect of climate change will slightly decrease the net effect of take-all disease in Ontario, Canada. Although the projected milder winters in Ontario will allow an increase in the saprotrophic survival of the *Ggt* fungus on crop debris and that there appears to be a trend for warmer summer that would increase the severity of take-all disease symptoms, the soil moisture is thought to decrease from increase evapotranspiration and the authors therefore conclude that climate change will lead to a decrease in take-all disease. Soil moisture is vital for root growth and a reduction in soil moisture can reduce root growth which in turn can reduce the chance of the root system coming into contact with fungal inoculum (Huisman, 1982), for example diseases caused by *Pythium* spp. are less severe in dry soil conditions (Garrett, 1938). On the other hand, there is also a prediction that warmer winters would favour other cereal soil-borne diseases, that infect their hosts during the autumn to winter months, such as eyespot (*Oculimacula acuformis* and *O. yallundae*) (West et al., 2012). The warmer soils are also thought to affect soil-borne viruses since vectors will be able to infect crops at an earlier growth stage; disease symptoms could again be exacerbated from heat stress (West et al., 2012).

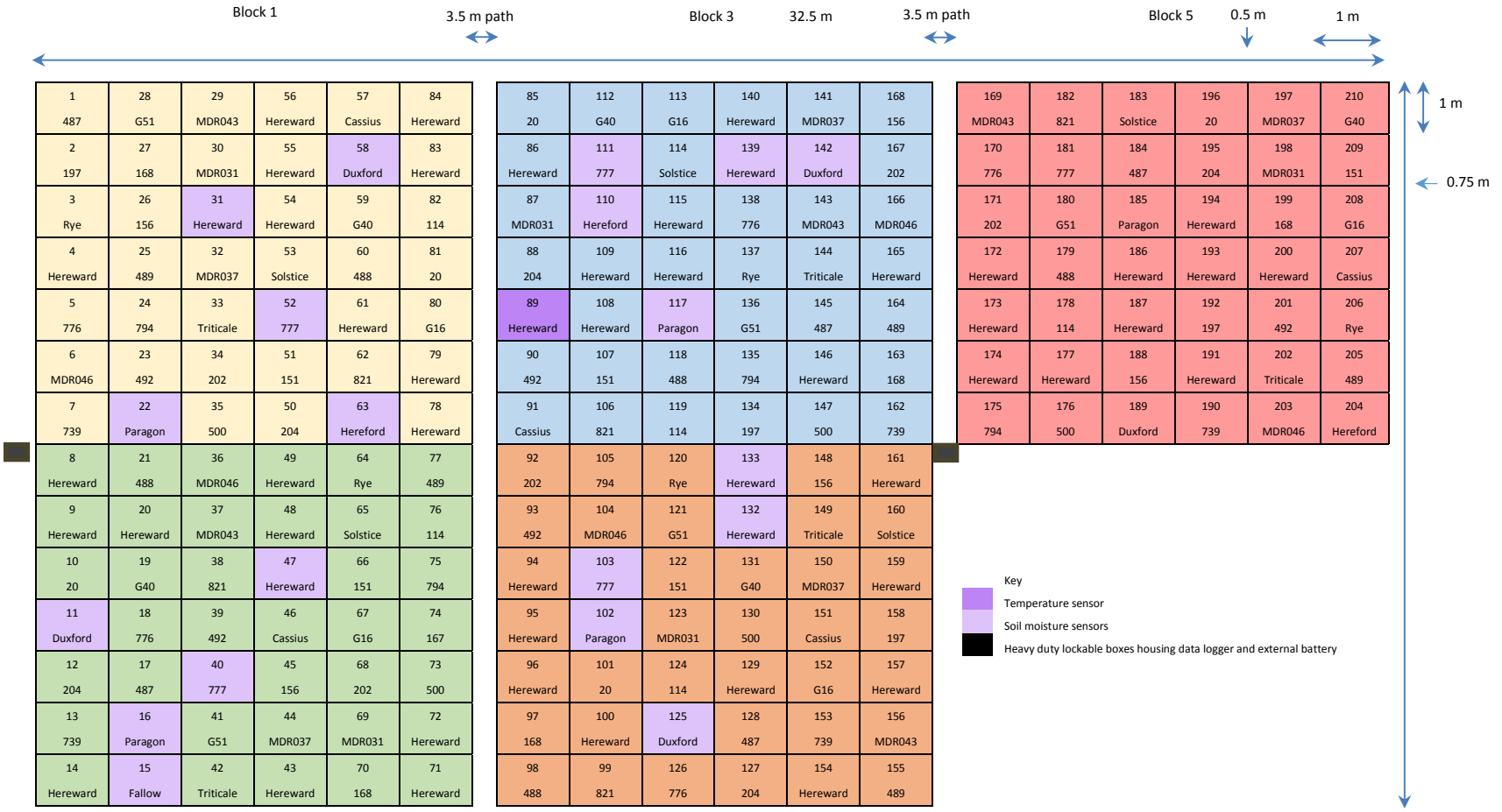
There is a need to model the severity of take-all disease in relation to environmental conditions such as weather conditions as well as estimate the projected climate change effects on the disease. This would help to forecast future take-all epidemics. The two years of field trial data collected within this PhD project would be valuable in this context due to the contrasting disease pressure years.

Field trial data from the Broadbalk long-term experiment, established in 1843 at RRes, would also be an invaluable tool in helping to establish the effects of weather and climate change on the disease. An integrated take-all control management strategy, as highlighted throughout this PhD, with the development of a take-all prediction model under varying environmental conditions, in relation to climate change models, will be required for the understanding and therefore control of the disease in the future.

# Appendices

**Appendix 3.1. Hexaploid field trial plans.**

Field trial: 2014/R/WW/1419




| | | | | | |
|---|---|---|---|---|---|
| 1<br>487 | 28<br>G51 | 29<br>MDR043 | 56<br>Hereward | 57<br>Cassius | 84<br>Hereward |
| 2<br>197 | 27<br>168 | 30<br>MDR031 | 55<br>Hereward | 58<br>Duxford | 83<br>Hereward |
| 3<br>Rye | 26<br>156 | 31<br>Hereward | 54<br>Hereward | 59<br>G40 | 82<br>114 |
| 4<br>Hereward | 25<br>489 | 32<br>MDR037 | 53<br>Solstice | 60<br>488 | 81<br>20 |
| 5<br>776 | 24<br>794 | 33<br>Triticale | 52<br>777 | 61<br>Hereward | 80<br>G16 |
| 6<br>MDR046 | 23<br>492 | 34<br>202 | 51<br>151 | 62<br>821 | 79<br>Hereward |
| 7<br>739 | 22<br>Paragon | 35<br>500 | 50<br>204 | 63<br>Hereford | 78<br>Hereward |
| 8<br>Hereward | 21<br>488 | 36<br>MDR046 | 49<br>Hereward | 64<br>Rye | 77<br>489 |
| 9<br>Hereward | 20<br>Hereward | 37<br>MDR043 | 48<br>Hereward | 65<br>Solstice | 76<br>114 |
| 10<br>20 | 19<br>G40 | 38<br>821 | 47<br>Hereward | 66<br>151 | 75<br>794 |
| 11<br>Duxford | 18<br>776 | 39<br>492 | 46<br>Cassius | 67<br>G16 | 74<br>167 |
| 12<br>204 | 17<br>487 | 40<br>777 | 45<br>156 | 68<br>202 | 73<br>500 |
| 13<br>739 | 16<br>Paragon | 41<br>G51 | 44<br>MDR037 | 69<br>MDR031 | 72<br>Hereward |
| 14<br>Hereward | 15<br>Fallow | 42<br>Triticale | 43<br>Hereward | 70<br>168 | 71<br>Hereward |

| | | | | | |
|---|---|---|---|---|---|
| 85<br>20 | 112<br>G40 | 113<br>G16 | 140<br>Hereward | 141<br>MDR037 | 168<br>156 |
| 86<br>Hereward | 111<br>777 | 114<br>Solstice | 139<br>Hereward | 142<br>Duxford | 167<br>202 |
| 87<br>MDR031 | 110<br>Hereford | 115<br>Hereward | 138<br>776 | 143<br>MDR043 | 166<br>MDR046 |
| 88<br>204 | 109<br>Hereward | 116<br>Hereward | 137<br>Rye | 144<br>Triticale | 165<br>Hereward |
| 89<br>Hereward | 108<br>Hereward | 117<br>Paragon | 136<br>G51 | 145<br>487 | 164<br>489 |
| 90<br>492 | 107<br>151 | 118<br>488 | 135<br>794 | 146<br>Hereward | 163<br>168 |
| 91<br>Cassius | 106<br>821 | 119<br>114 | 134<br>197 | 147<br>500 | 162<br>739 |
| 92<br>202 | 105<br>794 | 120<br>Rye | 133<br>Hereward | 148<br>156 | 161<br>Hereward |
| 93<br>492 | 104<br>MDR046 | 121<br>G51 | 132<br>Hereward | 149<br>Triticale | 160<br>Solstice |
| 94<br>Hereward | 103<br>777 | 122<br>151 | 131<br>G40 | 150<br>MDR037 | 159<br>Hereward |
| 95<br>Hereward | 102<br>Paragon | 123<br>MDR031 | 130<br>500 | 151<br>Cassius | 158<br>197 |
| 96<br>Hereward | 101<br>20 | 124<br>114 | 129<br>Hereward | 152<br>G16 | 157<br>Hereward |
| 97<br>168 | 100<br>Hereward | 125<br>Duxford | 128<br>487 | 153<br>739 | 156<br>MDR043 |
| 98<br>488 | 99<br>821 | 126<br>776 | 127<br>204 | 154<br>Hereward | 155<br>489 |

| | | | | | |
|---|---|---|---|---|---|
| 169<br>MDR043 | 182<br>821 | 183<br>Solstice | 196<br>20 | 197<br>MDR037 | 210<br>G40 |
| 170<br>776 | 181<br>777 | 184<br>487 | 195<br>204 | 198<br>MDR031 | 209<br>151 |
| 171<br>202 | 180<br>G51 | 185<br>Paragon | 194<br>Hereward | 199<br>168 | 208<br>G16 |
| 172<br>Hereward | 179<br>488 | 186<br>Hereward | 193<br>Hereward | 200<br>Hereward | 207<br>Cassius |
| 173<br>Hereward | 178<br>114 | 187<br>Hereward | 192<br>197 | 201<br>492 | 206<br>Rye |
| 174<br>Hereward | 177<br>Hereward | 188<br>156 | 191<br>Hereward | 202<br>Triticale | 205<br>489 |
| 175<br>794 | 176<br>500 | 189<br>Duxford | 190<br>739 | 203<br>MDR046 | 204<br>Hereford |

**Appendix 3.1. Continued.**

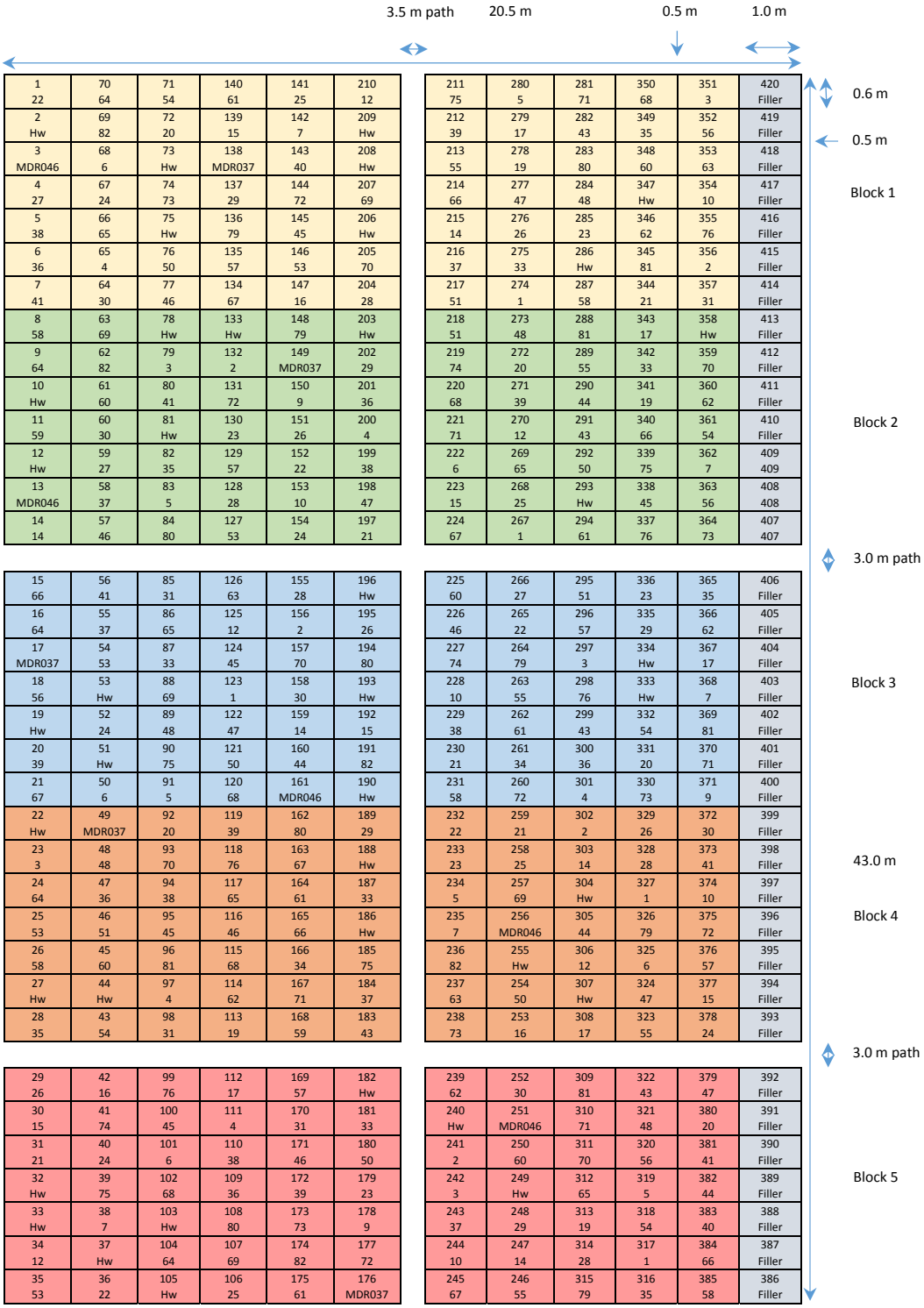
Field trial: 2015/R/WW/1510
Dummy plots: Hereward




| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 Solstice | 40 MDR043 | 41 197 | 80 MDR046 | 81 Dummy | 120 776 | 121 SA 3 | 160 Dummy | 161 489 | 200 Cassius | 201 Frument | 240 156 |
| 2 794 | 39 G51 | 42 487 | 79 G16 | 82 SA 1 | 119 Dummy | 122 Dummy | 159 739 | 162 Hereward | 199 488 | 202 204 | 239 SA 2 |
| 3 Triticale | 38 492 | 43 821 | 78 Hereward | 83 Duxford | 118 Hereward | 123 500 | 158 Hereward | 163 Paragon | 198 Hereward | 203 151 | 238 Hereford |
| 4 Dummy | 37 Rye | 44 20 | 77 168 | 84 G40 | 117 Hereward | 124 MDR037 | 157 777 | 164 114 | 197 202 | 204 MDR031 | 237 Dummy |
| 5 Rye | 36 MDR031 | 45 204 | 76 Hereward | 85 794 | 116 Hereward | 125 492 | 156 Dummy | 165 20 | 196 488 | 205 G16 | 236 Hereward |
| 6 Cassius | 35 776 | 46 Triticale | 75 151 | 86 Hereward | 115 Dummy | 126 Dummy | 155 Hereward | 166 202 | 195 Frument | 206 500 | 235 487 |
| 7 168 | 34 777 | 47 G51 | 74 Paragon | 87 MDR046 | 114 Dummy | 127 739 | 154 G40 | 167 197 | 194 MDR037 | 207 Dummy | 234 Duxford |
| 8 156 | 33 MDR043 | 48 114 | 73 Dummy | 88 Hereford | 113 SA 2 | 128 Hereward | 153 Solstice | 168 SA 1 | 193 821 | 208 Hereward | 233 489 |
| 9 821 | 32 Dummy | 49 Hereward | 72 114 | 89 G40 | 112 Cassius | 129 MDR037 | 152 Hereward | 169 Hereford | 192 Hereward | 209 487 | 232 776 |
| 10 492 | 31 Hereward | 50 Rye | 71 777 | 90 Frument | 111 Hereward | 130 489 | 151 20 | 170 Duxford | 191 Dummy | 210 MDR031 | 231 MDR046 |
| 11 739 | 30 151 | 51 Solstice | 70 SA 3 | 91 G16 | 110 Dummy | 131 SA 1 | 150 202 | 171 Triticale | 190 156 | 211 Dummy | 230 168 |
| 12 197 | 29 Hereward | 52 488 | 69 Paragon | 92 Dummy | 109 794 | 132 G51 | 149 MDR043 | 172 Hereward | 189 500 | 212 Dummy | 229 204 |
| 13 Duxford | 28 168 | 53 500 | 68 G16 | 93 Dummy | 108 Hereward | 133 Hereward | 148 204 | 173 777 | 188 Dummy | 213 Solstice | 228 G40 |
| 14 739 | 27 MDR043 | 54 489 | 67 Hereward | 94 Hereward | 107 202 | 134 Dummy | 147 Paragon | 174 776 | 187 SA 1 | 214 114 | 227 Rye |
| 15 Hereward | 26 MDR031 | 55 Hereward | 66 156 | 95 487 | 106 488 | 135 Dummy | 146 Hereward | 175 492 | 186 794 | 215 Cassius | 226 MDR046 |
| 16 151 | 25 MDR037 | 56 Dummy | 65 821 | 96 G51 | 105 Frument | 136 Hereford | 145 Triticale | 176 SA 3 | 185 197 | 216 Dummy | 225 20 |
| 17 Paragon | 24 Hereward | 57 739 | 64 492 | 97 Dummy | 104 202 | 137 G51 | 144 MDR031 | 177 197 | 184 SA 2 | 217 Dummy | 224 821 |
| 18 500 | 23 114 | 58 Duxford | 63 Dummy | 98 Hereward | 103 SA 3 | 138 794 | 143 Cassius | 178 MDR043 | 183 MDR037 | 218 Hereward | 223 168 |
| 19 204 | 22 20 | 59 Frument | 62 SA 1 | 99 Hereford | 102 Dummy | 139 G16 | 142 Hereward | 179 Dummy | 182 Rye | 219 776 | 222 489 |
| 20 Triticale | 21 Hereward | 60 487 | 61 Dummy | 100 Solstice | 101 777 | 140 156 | 141 Hereward | 180 MDR046 | 181 151 | 220 G40 | 221 488 |

**Appendix 4.1. *Triticum monococcum* MDR037 (S) X MDR046 (R) field trial plans.**

Field trial: 2014/R/WW/1418  Filler plots: Hereward (Hw)




| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 70 | 71 | 140 | 141 | 210 | 211 | 280 | 281 | 350 | 351 | 420 |
| 22 | 64 | 54 | 61 | 25 | 12 | 75 | 5 | 71 | 68 | 3 | Filler |
| 2 | 69 | 72 | 139 | 142 | 209 | 212 | 279 | 282 | 349 | 352 | 419 |
| Hw | 82 | 20 | 15 | 7 | Hw | 39 | 17 | 43 | 35 | 56 | Filler |
| 3 | 68 | 73 | 138 | 143 | 208 | 213 | 278 | 283 | 348 | 353 | 418 |
| MDR046 | 6 | Hw | MDR037 | 40 | Hw | 55 | 19 | 80 | 60 | 63 | Filler |
| 4 | 67 | 74 | 137 | 144 | 207 | 214 | 277 | 284 | 347 | 354 | 417 |
| 27 | 24 | 73 | 29 | 72 | 69 | 66 | 47 | 48 | Hw | 10 | Filler |
| 5 | 66 | 75 | 136 | 145 | 206 | 215 | 276 | 285 | 346 | 355 | 416 |
| 38 | 65 | Hw | 79 | 45 | Hw | 14 | 26 | 23 | 62 | 76 | Filler |
| 6 | 65 | 76 | 135 | 146 | 205 | 216 | 275 | 286 | 345 | 356 | 415 |
| 36 | 4 | 50 | 57 | 53 | 70 | 37 | 33 | Hw | 81 | 2 | Filler |
| 7 | 64 | 77 | 134 | 147 | 204 | 217 | 274 | 287 | 344 | 357 | 414 |
| 41 | 30 | 46 | 67 | 16 | 28 | 51 | 1 | 58 | 21 | 31 | Filler |
| 8 | 63 | 78 | 133 | 148 | 203 | 218 | 273 | 288 | 343 | 358 | 413 |
| 58 | 69 | Hw | Hw | 79 | Hw | 51 | 48 | 81 | 17 | Hw | Filler |
| 9 | 62 | 79 | 132 | 149 | 202 | 219 | 272 | 289 | 342 | 359 | 412 |
| 64 | 82 | 3 | 2 | MDR037 | 29 | 74 | 20 | 55 | 33 | 70 | Filler |
| 10 | 61 | 80 | 131 | 150 | 201 | 220 | 271 | 290 | 341 | 360 | 411 |
| Hw | 60 | 41 | 72 | 9 | 36 | 68 | 39 | 44 | 19 | 62 | Filler |
| 11 | 60 | 81 | 130 | 151 | 200 | 221 | 270 | 291 | 340 | 361 | 410 |
| 59 | 30 | Hw | 23 | 26 | 4 | 71 | 12 | 43 | 66 | 54 | Filler |
| 12 | 59 | 82 | 129 | 152 | 199 | 222 | 269 | 292 | 339 | 362 | 409 |
| Hw | 27 | 35 | 57 | 22 | 38 | 6 | 65 | 50 | 75 | 7 | 409 |
| 13 | 58 | 83 | 128 | 153 | 198 | 223 | 268 | 293 | 338 | 363 | 408 |
| MDR046 | 37 | 5 | 28 | 10 | 47 | 15 | 25 | Hw | 45 | 56 | 408 |
| 14 | 57 | 84 | 127 | 154 | 197 | 224 | 267 | 294 | 337 | 364 | 407 |
| 14 | 46 | 80 | 53 | 24 | 21 | 67 | 1 | 61 | 76 | 73 | 407 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 15 | 56 | 85 | 126 | 155 | 196 | 225 | 266 | 295 | 336 | 365 | 406 |
| 66 | 41 | 31 | 63 | 28 | Hw | 60 | 27 | 51 | 23 | 35 | Filler |
| 16 | 55 | 86 | 125 | 156 | 195 | 226 | 265 | 296 | 335 | 366 | 405 |
| 64 | 37 | 65 | 12 | 2 | 26 | 46 | 22 | 57 | 29 | 62 | Filler |
| 17 | 54 | 87 | 124 | 157 | 194 | 227 | 264 | 297 | 334 | 367 | 404 |
| MDR037 | 53 | 33 | 45 | 70 | 80 | 74 | 79 | 3 | Hw | 17 | Filler |
| 18 | 53 | 88 | 123 | 158 | 193 | 228 | 263 | 298 | 333 | 368 | 403 |
| 56 | Hw | 69 | 1 | 30 | Hw | 10 | 55 | 76 | Hw | 7 | Filler |
| 19 | 52 | 89 | 122 | 159 | 192 | 229 | 262 | 299 | 332 | 369 | 402 |
| Hw | 24 | 48 | 47 | 14 | 15 | 38 | 61 | 43 | 54 | 81 | Filler |
| 20 | 51 | 90 | 121 | 160 | 191 | 230 | 261 | 300 | 331 | 370 | 401 |
| 39 | Hw | 75 | 50 | 44 | 82 | 21 | 34 | 36 | 20 | 71 | Filler |
| 21 | 50 | 91 | 120 | 161 | 190 | 231 | 260 | 301 | 330 | 371 | 400 |
| 67 | 6 | 5 | 68 | MDR046 | Hw | 58 | 72 | 4 | 73 | 9 | Filler |
| 22 | 49 | 92 | 119 | 162 | 189 | 232 | 259 | 302 | 329 | 372 | 399 |
| Hw | MDR037 | 20 | 39 | 80 | 29 | 22 | 21 | 2 | 26 | 30 | Filler |
| 23 | 48 | 93 | 118 | 163 | 188 | 233 | 258 | 303 | 328 | 373 | 398 |
| 3 | 48 | 70 | 76 | 67 | Hw | 23 | 25 | 14 | 28 | 41 | Filler |
| 24 | 47 | 94 | 117 | 164 | 187 | 234 | 257 | 304 | 327 | 374 | 397 |
| 64 | 36 | 38 | 65 | 61 | 33 | 5 | 69 | Hw | 1 | 10 | Filler |
| 25 | 46 | 95 | 116 | 165 | 186 | 235 | 256 | 305 | 326 | 375 | 396 |
| 53 | 51 | 45 | 46 | 66 | Hw | 7 | MDR046 | 44 | 79 | 72 | Filler |
| 26 | 45 | 96 | 115 | 166 | 185 | 236 | 255 | 306 | 325 | 376 | 395 |
| 58 | 60 | 81 | 68 | 34 | 75 | 82 | Hw | 12 | 6 | 57 | Filler |
| 27 | 44 | 97 | 114 | 167 | 184 | 237 | 254 | 307 | 324 | 377 | 394 |
| Hw | Hw | 4 | 62 | 71 | 37 | 63 | 50 | Hw | 47 | 15 | Filler |
| 28 | 43 | 98 | 113 | 168 | 183 | 238 | 253 | 308 | 323 | 378 | 393 |
| 35 | 54 | 31 | 19 | 59 | 43 | 73 | 16 | 17 | 55 | 24 | Filler |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | 42 | 99 | 112 | 169 | 182 | 239 | 252 | 309 | 322 | 379 | 392 |
| 26 | 16 | 76 | 17 | 57 | Hw | 62 | 30 | 81 | 43 | 47 | Filler |
| 30 | 41 | 100 | 111 | 170 | 181 | 240 | 251 | 310 | 321 | 380 | 391 |
| 15 | 74 | 45 | 4 | 31 | 33 | Hw | MDR046 | 71 | 48 | 20 | Filler |
| 31 | 40 | 101 | 110 | 171 | 180 | 241 | 250 | 311 | 320 | 381 | 390 |
| 21 | 24 | 6 | 38 | 46 | 50 | 2 | 60 | 70 | 56 | 41 | Filler |
| 32 | 39 | 102 | 109 | 172 | 179 | 242 | 249 | 312 | 319 | 382 | 389 |
| Hw | 75 | 68 | 36 | 39 | 23 | 3 | Hw | 65 | 5 | 44 | Filler |
| 33 | 38 | 103 | 108 | 173 | 178 | 243 | 248 | 313 | 318 | 383 | 388 |
| Hw | 7 | Hw | 80 | 73 | 9 | 37 | 29 | 19 | 54 | 40 | Filler |
| 34 | 37 | 104 | 107 | 174 | 177 | 244 | 247 | 314 | 317 | 384 | 387 |
| 12 | Hw | 64 | 69 | 82 | 72 | 10 | 14 | 28 | 1 | 66 | Filler |
| 35 | 36 | 105 | 106 | 175 | 176 | 245 | 246 | 315 | 316 | 385 | 386 |
| 53 | 22 | Hw | 25 | 61 | MDR037 | 67 | 55 | 79 | 35 | 58 | Filler |

**Appendix 4.1. Continued.**

Field trial: 2015/R/WW/1512 Filler plots: JB Diego Hw: Hereward




| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1<br>77 | 80<br>21 | 81<br>76 | 160<br>7 | 161<br>26 | 240<br>63 | 241<br>59 | 320<br>74 | 321<br>46 | 400<br>12 | 401<br>35 | 480<br>Filler |
| 2<br>3 | 79<br>2 | 82<br>68 | 159<br>52 | 162<br>9 | 239<br>78 | 242<br>23 | 319<br>83 | 322<br>64 | 399<br>70 | 402<br>29 | 479<br>Filler |
| 3<br>22 | 78<br>Hw | 83<br>14 | 158<br>16 | 163<br>56 | 238<br>15 | 243<br>37 | 318<br>61 | 323<br>13 | 398<br>71 | 403<br>51 | 478<br>Filler |
| 4<br>4 | 77<br>28 | 84<br>44 | 157<br>10 | 164<br>Hw | 237<br>Hw | 244<br>54 | 317<br>79 | 324<br>6 | 397<br>82 | 404<br>80 | 477<br>Filler |
| 5<br>5 | 76<br>43 | 85<br>73 | 156<br>47 | 165<br>42 | 236<br>53 | 245<br>25 | 316<br>19 | 325<br>69 | 396<br>Hw | 405<br>40 | 476<br>Filler |
| 6<br>65 | 75<br>27 | 86<br>11 | 155<br>Hw | 166<br>55 | 235<br>38 | 246<br>31 | 315<br>39 | 326<br>MDR037 | 395<br>34 | 406<br>24 | 475<br>Filler |
| 7<br>58 | 74<br>20 | 87<br>81 | 154<br>33 | 167<br>1 | 234<br>36 | 247<br>45 | 314<br>Hw | 327<br>Hw | 394<br>50 | 407<br>62 | 474<br>Filler |
| 8<br>41 | 73<br>MDR046 | 88<br>67 | 153<br>17 | 168<br>72 | 233<br>30 | 248<br>60 | 313<br>48 | 328<br>75 | 393<br>66 | 408<br>57 | 473<br>Filler |
| 9<br>42 | 72<br>81 | 89<br>29 | 152<br>38 | 169<br>72 | 232<br>56 | 249<br>73 | 312<br>30 | 329<br>82 | 392<br>MD046 | 409<br>19 | 472<br>Filler |
| 10<br>10 | 71<br>Hw | 90<br>40 | 151<br>25 | 170<br>74 | 231<br>34 | 250<br>22 | 311<br>72 | 330<br>79 | 391<br>58 | 410<br>35 | 471<br>Filler |
| 11<br>41 | 70<br>Hw | 91<br>15 | 150<br>75 | 171<br>27 | 230<br>47 | 251<br>52 | 310<br>33 | 331<br>63 | 390<br>64 | 411<br>44 | 470<br>Filler |
| 12<br>69 | 69<br>76 | 92<br>50 | 149<br>55 | 172<br>67 | 229<br>Hw | 252<br>59 | 309<br>36 | 332<br>3 | 389<br>31 | 412<br>60 | 469<br>Filler |
| 13<br>11 | 68<br>28 | 93<br>21 | 148<br>62 | 173<br>66 | 228<br>78 | 253<br>2 | 308<br>61 | 333<br>57 | 388<br>Hw | 413<br>MDR037 | 468<br>Filler |
| 14<br>68 | 67<br>14 | 94<br>48 | 147<br>Hw | 174<br>16 | 227<br>5 | 254<br>12 | 307<br>80 | 334<br>26 | 387<br>54 | 414<br>45 | 467<br>Filler |
| 15<br>51 | 66<br>70 | 95<br>53 | 146<br>4 | 175<br>65 | 226<br>17 | 255<br>46 | 306<br>Hw | 335<br>71 | 386<br>7 | 415<br>23 | 466<br>Filler |
| 16<br>6 | 65<br>13 | 96<br>77 | 145<br>Hw | 176<br>9 | 225<br>43 | 256<br>39 | 305<br>20 | 336<br>37 | 385<br>24 | 416<br>83 | 465<br>Filler |
| 17<br>17 | 64<br>40 | 97<br>47 | 144<br>72 | 177<br>37 | 224<br>70 | 257<br>20 | 304<br>MDR046 | 337<br>36 | 384<br>27 | 417<br>16 | 464<br>Filler |
| 18<br>7 | 63<br>24 | 98<br>31 | 143<br>12 | 178<br>28 | 223<br>57 | 258<br>69 | 303<br>42 | 338<br>48 | 383<br>30 | 418<br>4 | 463<br>Filler |
| 19<br>66 | 62<br>Hw | 99<br>76 | 142<br>43 | 179<br>2 | 222<br>23 | 259<br>5 | 302<br>10 | 339<br>58 | 382<br>54 | 419<br>39 | 462<br>Filler |
| 20<br>75 | 61<br>74 | 100<br>46 | 141<br>45 | 180<br>Hw | 221<br>Hw | 260<br>14 | 301<br>65 | 340<br>MDR037 | 381<br>Hw | 420<br>29 | 461<br>Filler |
| 21<br>1 | 60<br>15 | 101<br>68 | 140<br>50 | 181<br>35 | 220<br>83 | 261<br>80 | 300<br>38 | 341<br>52 | 380<br>Hw | 421<br>34 | 460<br>Filler |
| 22<br>71 | 59<br>79 | 102<br>77 | 139<br>Hw | 182<br>82 | 219<br>81 | 262<br>62 | 299<br>53 | 342<br>67 | 379<br>13 | 422<br>59 | 459<br>Filler |
| 23<br>Hw | 58<br>60 | 103<br>Hw | 138<br>78 | 183<br>63 | 218<br>41 | 263<br>61 | 298<br>Hw | 343<br>6 | 378<br>56 | 423<br>73 | 458<br>Filler |
| 24<br>22 | 57<br>9 | 104<br>25 | 137<br>44 | 184<br>3 | 217<br>51 | 264<br>11 | 297<br>64 | 344<br>21 | 377<br>55 | 424<br>19 | 457<br>Filler |
| 25<br>42 | 56<br>68 | 105<br>Hw | 136<br>41 | 185<br>4 | 216<br>11 | 265<br>58 | 296<br>13 | 345<br>45 | 376<br>67 | 425<br>23 | 456<br>Filler |
| 26<br>55 | 55<br>20 | 106<br>24 | 135<br>43 | 186<br>62 | 215<br>80 | 266<br>61 | 295<br>44 | 346<br>30 | 375<br>Hw | 426<br>53 | 455<br>Filler |
| 27<br>17 | 54<br>Hw | 107<br>79 | 134<br>27 | 187<br>48 | 214<br>77 | 267<br>50 | 294<br>65 | 347<br>73 | 374<br>25 | 427<br>78 | 454<br>Filler |
| 28<br>MDR046 | 53<br>5 | 108<br>7 | 133<br>81 | 188<br>57 | 213<br>Hw | 268<br>9 | 293<br>34 | 348<br>16 | 373<br>22 | 428<br>64 | 453<br>Filler |
| 29<br>74 | 52<br>72 | 109<br>63 | 132<br>31 | 189<br>71 | 212<br>39 | 269<br>36 | 292<br>29 | 349<br>54 | 372<br>Hw | 429<br>3 | 452<br>Filler |
| 30<br>66 | 51<br>47 | 110<br>Hw | 131<br>Hw | 190<br>46 | 211<br>51 | 270<br>6 | 291<br>1 | 350<br>28 | 371<br>35 | 430<br>60 | 451<br>Filler |
| 31<br>37 | 50<br>14 | 111<br>75 | 130<br>59 | 191<br>19 | 210<br>69 | 271<br>83 | 290<br>Hw | 351<br>38 | 370<br>10 | 431<br>21 | 450<br>Filler |
| 32<br>Hw | 49<br>MDR037 | 112<br>52 | 129<br>40 | 192<br>12 | 209<br>56 | 272<br>70 | 289<br>15 | 352<br>82 | 369<br>76 | 432<br>2 | 449<br>Filler |
| 33<br>57 | 48<br>25 | 113<br>60 | 128<br>83 | 193<br>71 | 208<br>65 | 273<br>52 | 288<br>28 | 353<br>Hw | 368<br>43 | 433<br>54 | 448<br>Filler |
| 34<br>17 | 47<br>13 | 114<br>46 | 127<br>66 | 194<br>5 | 207<br>11 | 274<br>36 | 287<br>59 | 354<br>27 | 367<br>73 | 434<br>15 | 447<br>Filler |
| 35<br>MDR037 | 46<br>Hw | 115<br>72 | 126<br>67 | 195<br>9 | 206<br>29 | 275<br>Hw | 286<br>19 | 355<br>80 | 366<br>7 | 435<br>20 | 446<br>Filler |
| 36<br>68 | 45<br>38 | 116<br>39 | 125<br>12 | 196<br>62 | 205<br>Hw | 276<br>51 | 285<br>40 | 356<br>21 | 365<br>41 | 436<br>70 | 445<br>Filler |
| 37<br>75 | 44<br>76 | 117<br>24 | 124<br>Hw | 197<br>81 | 204<br>10 | 277<br>6 | 284<br>3 | 357<br>45 | 364<br>Hw | 437<br>22 | 444<br>Filler |
| 38<br>74 | 43<br>MDR046 | 118<br>56 | 123<br>Hw | 198<br>37 | 203<br>44 | 278<br>78 | 283<br>58 | 358<br>69 | 363<br>4 | 438<br>50 | 443<br>Filler |
| 39<br>42 | 42<br>48 | 119<br>55 | 122<br>64 | 199<br>35 | 202<br>82 | 279<br>2 | 282<br>Hw | 359<br>16 | 362<br>77 | 439<br>30 | 442<br>Filler |
| 40<br>63 | 41<br>53 | 120<br>1 | 121<br>47 | 200<br>31 | 201<br>79 | 280<br>61 | 281<br>Hw | 360<br>14 | 361<br>23 | 440<br>34 | 441<br>Filler |



**Appendix 4.2. *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population lines multiplied in the glasshouse to provide seed for field trials.**

| Treatment | 14/R/WW/1418 | | 15/R/WW/1512 | |
|---|---|---|---|---|
| Control species | Number of replicates | Multiplied in the glasshouse (Yes/No) | Number of replicates | Multiplied in the glasshouse (Yes/No) |
| MDR037 | 5 | No | 5 | No |
| MDR046 | 5 | No | 5 | No |
| Hereward | 40 | No | 41 | No |
| ***Triticum monococcum* MDR037 X MDR046 lines** | | | | |
| 1 | 5 | No | 5 | Yes |
| 2 | 5 | No | 5 | No |
| 3 | 5 | No | 5 | Yes |
| 4 | 5 | No | 5 | No |
| 5 | 5 | No | 5 | Yes |
| 6 | 5 | No | 5 | Yes |
| 7 | 5 | No | 5 | No |
| 9 | 3 | No | 5 | Yes |
| 10 | 5 | No | 5 | No |
| 11 | 0 | Yes | 5 | No |
| 12 | 5 | No | 5 | Yes |
| 13 | 0 | Yes | 5 | No |
| 14 | 5 | No | 5 | No |
| 15 | 5 | No | 5 | No |
| 16 | 3 | No | 5 | Yes |
| 17 | 5 | No | 5 | Yes |
| 19 | 4 | No | 5 | Yes |
| 20 | 5 | No | 5 | No |
| 21 | 5 | No | 5 | Yes |
| 22 | 5 | No | 5 | Yes |
| 23 | 5 | No | 5 | Yes |
| 24 | 5 | No | 5 | No |
| 25 | 4 | No | 5 | No |
| 26 | 5 | No | 2 | Yes |
| 27 | 3 | No | 5 | No |
| 28 | 5 | No | 5 | Yes |
| 29 | 5 | No | 5 | Yes |
| 30 | 5 | No | 5 | Yes |
| 31 | 4 | No | 5 | No |
| 33 | 5 | No | 2 | Yes |
| 34 | 2 | No | 5 | No |
| 35 | 5 | No | 5 | No |
| 36 | 5 | No | 5 | Yes |
| 37 | 5 | No | 5 | Yes |
| 38 | 5 | No | 5 | Yes |

**Appendix 4.2. Continued.**

| Treatment | 14/R/WW/1418 | | 15/R/WW/1512 | |
|---|---|---|---|---|
| Control species | Number of replicates | Multiplied in the glasshouse (Yes/No) | Number of replicates | Multiplied in the glasshouse (Yes/No) |
| ***Triticum monococcum* MDR037 X MDR046 lines** | | | | |
| 39 | 5 | No | 5 | Yes |
| 40 | 2 | No | 5 | No |
| 41 | 5 | No | 5 | Yes |
| 42 | 0 | Yes | 5 | No |
| 43 | 5 | No | 5 | Yes |
| 44 | 4 | No | 5 | Yes |
| 45 | 5 | No | 5 | No |
| 46 | 5 | No | 5 | Yes |
| 47 | 5 | No | 5 | Yes |
| 48 | 5 | No | 5 | No |
| 50 | 5 | No | 5 | Yes |
| 51 | 4 | No | 5 | No |
| 52 | 0 | Yes | 5 | No |
| 53 | 5 | No | 5 | No |
| 54 | 5 | No | 5 | No |
| 55 | 5 | No | 5 | No |
| 56 | 4 | No | 5 | Yes |
| 57 | 5 | No | 5 | Yes |
| 58 | 5 | No | 5 | No |
| 59 | 2 | No | 5 | No |
| 60 | 5 | No | 5 | No |
| 61 | 5 | No | 5 | No |
| 62 | 5 | No | 5 | No |
| 63 | 3 | No | 5 | No |
| 64 | 5 | No | 5 | Yes |
| 65 | 5 | No | 5 | Yes |
| 66 | 5 | No | 5 | No |
| 67 | 5 | No | 5 | Yes |
| 68 | 5 | No | 5 | Yes |
| 69 | 5 | No | 5 | Yes |
| 70 | 5 | No | 5 | No |
| 71 | 5 | No | 5 | No |
| 72 | 5 | No | 5 | No |
| 73 | 5 | No | 5 | No |
| 74 | 3 | No | 5 | Yes |
| 75 | 5 | No | 5 | No |
| 76 | 5 | No | 5 | No |
| 77 | 0 | Yes | 5 | No |
| 78 | 0 | Yes | 5 | No |
| 79 | 5 | No | 5 | No |
| 80 | 5 | No | 5 | Yes |
| 81 | 5 | No | 5 | Yes |
| 82 | 5 | No | 5 | Yes |
| 83 | 0 | Yes | 5 | No |

**Appendix 4.3. Establishment of the flooded *Triticum monococcum* MDR037 (S) X MDR046 (R) 2013-2014 field trial.**

Field trial: 2014/R/WW/1418

| Germination key | |
|---|---|
| Good | 41-80 plants |
| Medium | 21-40 plants |
| Poor | 6-20 plants |
| Very poor | <6 plants |
| Empty | Flooding event |
| Empty | Rodent infestation |

| | | | | | |
|---|---|---|---|---|---|
| 1<br>22 | 70<br>64 | 71<br>54 | 140<br>61 | 141<br>25 | 210<br>12 |
| 2<br>Hw | 69<br>82 | 72<br>20 | 139<br>15 | 142<br>7 | 209<br>Hw |
| 3<br>MDR046 | 68<br>6 | 73<br>Hw | 138<br>MDR037 | 143<br>40 | 208<br>Hw |
| 4<br>27 | 67<br>24 | 74<br>73 | 137<br>29 | 144<br>72 | 207<br>69 |
| 5<br>38 | 66<br>65 | 75<br>Hw | 136<br>79 | 145<br>45 | 206<br>Hw |
| 6<br>36 | 65<br>4 | 76<br>50 | 135<br>57 | 146<br>53 | 205<br>70 |
| 7<br>41 | 64<br>30 | 77<br>46 | 134<br>67 | 147<br>16 | 204<br>28 |
| 8<br>58 | 63<br>69 | 78<br>Hw | 133<br>Hw | 148<br>79 | 203<br>Hw |
| 9<br>64 | 62<br>82 | 79<br>3 | 132<br>2 | 149<br>MDR037 | 202<br>29 |
| 10<br>Hw | 61<br>60 | 80<br>41 | 131<br>72 | 150<br>9 | 201<br>36 |
| 11<br>59 | 60<br>30 | 81<br>Hw | 130<br>23 | 151<br>26 | 200<br>4 |
| 12<br>Hw | 59<br>27 | 82<br>35 | 129<br>57 | 152<br>22 | 199<br>38 |
| 13<br>MDR046 | 58<br>37 | 83<br>5 | 128<br>28 | 153<br>10 | 198<br>47 |
| 14<br>14 | 57<br>46 | 84<br>80 | 127<br>53 | 154<br>24 | 197<br>21 |

| | | | | | |
|---|---|---|---|---|---|
| 211<br>75 | 280<br>5 | 281<br>71 | 350<br>68 | 351<br>3 | 420<br>Filler |
| 212<br>39 | 279<br>17 | 282<br>43 | 349<br>35 | 352<br>56 | 419<br>Filler |
| 213<br>55 | 278<br>19 | 283<br>80 | 348<br>60 | 353<br>63 | 418<br>Filler |
| 214<br>66 | 277<br>47 | 284<br>48 | 347<br>Hw | 354<br>10 | 417<br>Filler |
| 215<br>14 | 276<br>26 | 285<br>23 | 346<br>62 | 355<br>76 | 416<br>Filler |
| 216<br>37 | 275<br>33 | 286<br>Hw | 345<br>81 | 356<br>2 | 415<br>Filler |
| 217<br>51 | 274<br>1 | 287<br>58 | 344<br>21 | 357<br>31 | 414<br>Filler |
| 218<br>51 | 273<br>48 | 288<br>81 | 343<br>17 | 358<br>Hw | 413<br>Filler |
| 219<br>74 | 272<br>20 | 289<br>55 | 342<br>33 | 359<br>70 | 412<br>Filler |
| 220<br>68 | 271<br>39 | 290<br>44 | 341<br>19 | 360<br>62 | 411<br>Filler |
| 221<br>71 | 270<br>12 | 291<br>43 | 340<br>66 | 361<br>54 | 410<br>Filler |
| 222<br>6 | 269<br>65 | 292<br>50 | 339<br>75 | 362<br>7 | 409<br>409 |
| 223<br>15 | 268<br>25 | 293<br>Hw | 338<br>45 | 363<br>56 | 408<br>408 |
| 224<br>67 | 267<br>1 | 294<br>61 | 337<br>76 | 364<br>73 | 407<br>407 |

| | | | | | |
|---|---|---|---|---|---|
| 15<br>66 | 56<br>41 | 85<br>31 | 126<br>63 | 155<br>28 | 196<br>Hw |
| 16<br>64 | 55<br>37 | 86<br>65 | 125<br>12 | 156<br>2 | 195<br>26 |
| 17<br>MDR037 | 54<br>53 | 87<br>33 | 124<br>45 | 157<br>70 | 194<br>80 |
| 18<br>56 | 53<br>Hw | 88<br>69 | 123<br>1 | 158<br>30 | 193<br>Hw |
| 19<br>Hw | 52<br>24 | 89<br>48 | 122<br>47 | 159<br>14 | 192<br>15 |
| 20<br>39 | 51<br>Hw | 90<br>75 | 121<br>50 | 160<br>44 | 191<br>82 |
| 21<br>67 | 50<br>6 | 91<br>5 | 120<br>68 | 161<br>MDR046 | 190<br>Hw |
| 22<br>Hw | 49<br>MDR037 | 92<br>20 | 119<br>39 | 162<br>80 | 189<br>29 |
| 23<br>3 | 48<br>48 | 93<br>70 | 118<br>76 | 163<br>67 | 188<br>Hw |
| 24<br>64 | 47<br>36 | 94<br>38 | 117<br>65 | 164<br>61 | 187<br>33 |
| 25<br>53 | 46<br>51 | 95<br>45 | 116<br>46 | 165<br>66 | 186<br>Hw |
| 26<br>58 | 45<br>60 | 96<br>81 | 115<br>68 | 166<br>34 | 185<br>75 |
| 27<br>Hw | 44<br>Hw | 97<br>4 | 114<br>62 | 167<br>71 | 184<br>37 |
| 28<br>35 | 43<br>54 | 98<br>31 | 113<br>19 | 168<br>59 | 183<br>43 |

| | | | | | |
|---|---|---|---|---|---|
| 225<br>60 | 266<br>27 | 295<br>51 | 336<br>23 | 365<br>35 | 406<br>Filler |
| 226<br>46 | 265<br>22 | 296<br>57 | 335<br>29 | 366<br>62 | 405<br>Filler |
| 227<br>74 | 264<br>79 | 297<br>3 | 334<br>Hw | 367<br>17 | 404<br>Filler |
| 228<br>10 | 263<br>55 | 298<br>76 | 333<br>Hw | 368<br>7 | 403<br>Filler |
| 229<br>38 | 262<br>61 | 299<br>43 | 332<br>54 | 369<br>81 | 402<br>Filler |
| 230<br>21 | 261<br>34 | 300<br>36 | 331<br>20 | 370<br>71 | 401<br>Filler |
| 231<br>58 | 260<br>72 | 301<br>4 | 330<br>73 | 371<br>9 | 400<br>Filler |
| 232<br>22 | 259<br>21 | 302<br>2 | 329<br>26 | 372<br>30 | 399<br>Filler |
| 233<br>23 | 258<br>25 | 303<br>14 | 328<br>28 | 373<br>41 | 398<br>Filler |
| 234<br>5 | 257<br>69 | 304<br>Hw | 327<br>1 | 374<br>10 | 397<br>Filler |
| 235<br>7 | 256<br>MDR046 | 305<br>44 | 326<br>79 | 375<br>72 | 396<br>Filler |
| 236<br>82 | 255<br>Hw | 306<br>12 | 325<br>6 | 376<br>57 | 395<br>Filler |
| 237<br>63 | 254<br>50 | 307<br>Hw | 324<br>47 | 377<br>15 | 394<br>Filler |
| 238<br>73 | 253<br>16 | 308<br>17 | 323<br>55 | 378<br>24 | 393<br>Filler |

| | | | | | |
|---|---|---|---|---|---|
| 29<br>26 | 42<br>16 | 99<br>76 | 112<br>17 | 169<br>57 | 182<br>Hw |
| 30<br>15 | 41<br>74 | 100<br>45 | 111<br>4 | 170<br>31 | 181<br>33 |
| 31<br>21 | 40<br>24 | 101<br>6 | 110<br>38 | 171<br>46 | 180<br>50 |
| 32<br>Hw | 39<br>75 | 102<br>68 | 109<br>36 | 172<br>39 | 179<br>23 |
| 33<br>Hw | 38<br>7 | 103<br>Hw | 108<br>80 | 173<br>73 | 178<br>9 |
| 34<br>12 | 37<br>Hw | 104<br>64 | 107<br>69 | 174<br>82 | 177<br>72 |
| 35<br>53 | 36<br>22 | 105<br>Hw | 106<br>25 | 175<br>61 | 176<br>MDR037 |

| | | | | | |
|---|---|---|---|---|---|
| 239<br>62 | 252<br>30 | 309<br>81 | 322<br>43 | 379<br>47 | 392<br>Filler |
| 240<br>Hw | 251<br>MDR046 | 310<br>71 | 321<br>48 | 380<br>20 | 391<br>Filler |
| 241<br>2 | 250<br>60 | 311<br>70 | 320<br>56 | 381<br>41 | 390<br>Filler |
| 242<br>3 | 249<br>Hw | 312<br>65 | 319<br>5 | 382<br>44 | 389<br>Filler |
| 243<br>37 | 248<br>29 | 313<br>19 | 318<br>54 | 383<br>40 | 388<br>Filler |
| 244<br>10 | 247<br>14 | 314<br>28 | 317<br>1 | 384<br>66 | 387<br>Filler |
| 245<br>67 | 246<br>55 | 315<br>79 | 316<br>35 | 385<br>58 | 386<br>Filler |

**Appendix 4.4. Eighteen *Ggt* isolates used in the sand maizemeal inoculum for screening the *Triticum monococcum* accessions to take-all in a pot bioassay (*Ggt* isolates were provided and characterised by Gail Canning).**

| Isolate | Rothamsted Field | Year collected | Type A/B | Sensitivity (S) / insensitivity (IS) to silthiofam | Morphology |
|---|---|---|---|---|---|
| 7 | New Zealand | 2012 | A | IS | White |
| 8 | New Zealand | 2012 | B | S | White |
| 13 | New Zealand | 2012 | A | IS | Dark brown |
| 14 | New Zealand | 2012 | B | S | Light brown; Fast growing |
| 15 | New Zealand | 2012 | B | S | Dark brown |
| 16 | New Zealand | 2012 | B | S | Light brown |
| 21 | New Zealand | 2012 | A | IS | Dark brown |
| 23 | New Zealand | 2012 | B | S | Dark brown |
| 25 | New Zealand | 2012 | B | S | Brown |
| 36 | New Zealand | 2012 | A | IS | Dark brown |
| 44 | New Zealand | 2012 | B | S | Light brown |
| 45 | New Zealand | 2012 | B | S | Dark brown |
| 49 | New Zealand | 2012 | A | S | Dark brown; Fast growing |
| 53 | New Zealand | 2012 | A | IS | White |
| 56 | New Zealand | 2012 | A | S | Dark brown; Fast growing |
| 61 | New Zealand | 2012 | A | S | Dark brown; Fast growing |
| 62 | New Zealand | 2012 | A | IS | White |
| 66 | New Zealand | 2012 | A | IS | White |

**Appendix 4.5. *Triticum monococcum* accessions screened in a pot bioassay for susceptibility to take-all by Gail Canning.**

| ***Triticum monococcum* accession** | **Cultivar** | **Country of origin** | **Year collected** | **Donor institute** |
|---|---|---|---|---|
| MDR001 | *flavescens* | Algeria | - | JIC |
| MDR002 | *atriarstatum* | Balkans | - | JIC |
| MDR025 | *macedonicum; pseudoflavescens* | Ukraine | 1923 | VIR |
| MDR026 | *pseudomacedonicum* | Ukraine | 1923 | VIR |
| MDR031 | *monococcum; macedonicum* | Turkey | 1927 | VIR |
| MDR035 | *flavescens; vulgare* | Austria | 1930 | VIR |
| MDR037 | *macedonicum* | Armenia | 1934 | VIR |
| MDR040 | *vulgare; macedonicum* | Bulgaria | 1940 | VIR |
| MDR043 | *vulgare* | Greece | 1950 | VIR |
| MDR044 | *hornemannii* | Turkey | 1965 | VIR |
| MDR045 | *vulgare* | Denmark | 1970 | VIR |
| MDR046 | *atriaristatum; macedonicum* | Romania | 1970 | VIR |
| MDR047 | *macedonicum; vulgare* | Hungary | 1970 | VIR |
| MDR050 | - | Italy | - | VIR |
| MDR217 | 1277 | Turkey | - | USDA |
| MDR218 | 2592 | Turkey | - | USDA |
| MDR222 | 3281 | Turkey | - | USDA |
| MDR227 | Einkorn | United States | - | USDA |
| MDR228 | 2497 | Turkey | - | USDA |
| MDR229 | 3962 | Spain | - | USDA |
| MDR232 | *nigricultum* | Yugoslavia | - | USDA |

**Appendix 4.5. Continued.**

| *Triticum monococcum* accession | Cultivar | Country of origin | Year collected | Donor institute |
|---|---|---|---|---|
| MDR236 | I-1-1914 | Hungary | - | RRES |
| MDR243 | 2934 | Romania | - | RRES |
| MDR244 | K930 | Morocco | - | RRES |
| MDR258 | Einkorn | Israel | - | RRES |
| MDR261 | G2886 | Iraq | - | RRES |
| MDR264 | G2900 | Turkey | - | RRES |
| MDR279 | G2944 | Turkey | - | RRES |
| MDR280 | G2946 | Turkey | - | RRES |
| MDR286 | 84TK154-034 | Turkey | - | RRES |
| MDR303 | T-1600. | Spain | - | RRES |
| MDR306 | 957 | Yugoslavia | - | RRES |
| MDR308 | DV92 | Italy | - | - |
| MDR650 | PI355520 | Iran | - | ACFPG |

JIC = John Innes Centre

VIR = Vavilow Institute

USDA = United States Department of Agriculture

RRES = Rothamsted Research

ACFPG = Australian Centre for Plant Functional Genomics

**Appendix 4.6. Susceptibility of *Triticum monococcum* accessions and control varieties to take-all disease in a pot bioassay, assessed by Gail Canning.**

| Cereal genotype | Logit percentage of take-all infected roots (back transformed means) | | Number of roots/plant | Number of take-all infected roots/plant |
|---|---|---|---|---|
| **Controls** | | | | |
| Hereward negative | -2.203 | (0.71) | 5.31 | 0.20 |
| Rye | -1.132 | (8.91) | 8.22 | 1.54 |
| Paragon | -0.689 | (19.65) | 7.44 | 1.98 |
| Duxford | -0.454 | (28.24) | 7.26 | 2.42 |
| Triticale | -0.362 | (32.15) | 9.86 | 3.34 |
| Hereford | -0.350 | (32.70) | 6.50 | 2.66 |
| Hereward | -0.324 | (33.83) | 5.39 | 2.10 |
| ***Triticum monococcum* accessions** | | | | |
| MDR043 | -0.629 | (21.63) | 5.99 | 1.72 |
| MDR261 | -0.561 | (24.08) | 6.41 | 2.18 |
| MDR232 | -0.522 | (25.53) | 6.49 | 1.93 |
| MDR650 (PI355520) | -0.516 | (25.77) | 7.55 | 2.24 |
| MDR050 | -0.483 | (27.09) | 5.74 | 1.85 |
| MDR306 | -0.477 | (27.31) | 5.80 | 2.46 |
| MDR046 | -0.457 | (28.13) | 6.81 | 2.27 |
| MDR031 | -0.449 | (28.43) | 6.32 | 2.37 |
| MDR026 | -0.434 | (29.05) | 7.52 | 2.56 |
| MDR025 | -0.361 | (32.20) | 6.88 | 2.52 |
| MDR047 | -0.340 | (33.14) | 7.78 | 2.96 |
| MDR218 | -0.316 | (34.22) | 5.56 | 2.24 |
| MDR280 | -0.301 | (34.89) | 6.82 | 2.74 |
| MDR035 | -0.282 | (35.75) | 6.46 | 2.54 |
| MDR002 | -0.276 | (36.04) | 6.48 | 2.48 |
| MDR045 | -0.263 | (36.63) | 6.88 | 2.62 |
| MDR264 | -0.259 | (36.83) | 5.40 | 2.22 |
| MDR222 | -0.249 | (37.32) | 5.88 | 2.46 |
| MDR229 | -0.241 | (37.66) | 6.96 | 2.86 |
| MDR217 | -0.220 | (38.66) | 6.49 | 2.68 |
| MDR303 | -0.207 | (39.29) | 4.57 | 1.97 |
| MDR227 | -0.197 | (39.78) | 7.33 | 3.01 |
| MDR001 | -0.177 | (40.76) | 6.82 | 2.93 |
| MDR244 | -0.134 | (42.84) | 6.99 | 3.16 |
| MDR286 | -0.131 | (42.98) | 6.05 | 2.75 |
| MDR258 | -0.114 | (43.81) | 7.17 | 3.40 |
| MDR228 | -0.111 | (43.99) | 6.22 | 2.88 |
| MDR236 | -0.105 | (44.26) | 7.10 | 3.38 |
| MDR308 | -0.101 | (44.46) | 6.77 | 3.04 |

**Appendix 4.6. Continued.**

| Cereal genotype | Logit percentage of take-all infected roots (back transformed means) | | Number of roots/plant | Number of take-all infected roots/plant |
|---|---|---|---|---|
| ***Triticum monococcum* accessions** | | | | |
| MDR279 | -0.101 | (44.49) | 5.72 | 2.69 |
| MDR040 | -0.081 | (45.47) | 6.56 | 3.25 |
| MDR044 | -0.045 | (47.27) | 7.03 | 3.48 |
| MDR243 | -0.023 | (48.36) | 6.86 | 3.36 |
| MDR037 | 0.051 | (52.03) | 8.02 | 4.20 |
| d.f. | 40 | | 40 | 40 |
| SED | 0.23 | | 0.31 | 0.52 |
| F probability | <0.001 | | <0.001 | <0.001 |
| Grand mean | -0.357 | (41.08) | 6.67 | 2.58 |

**Appendix 5.1. DNA extraction solution recipes.**

**CTAB buffer:** Prepare in 1 litre bottle.

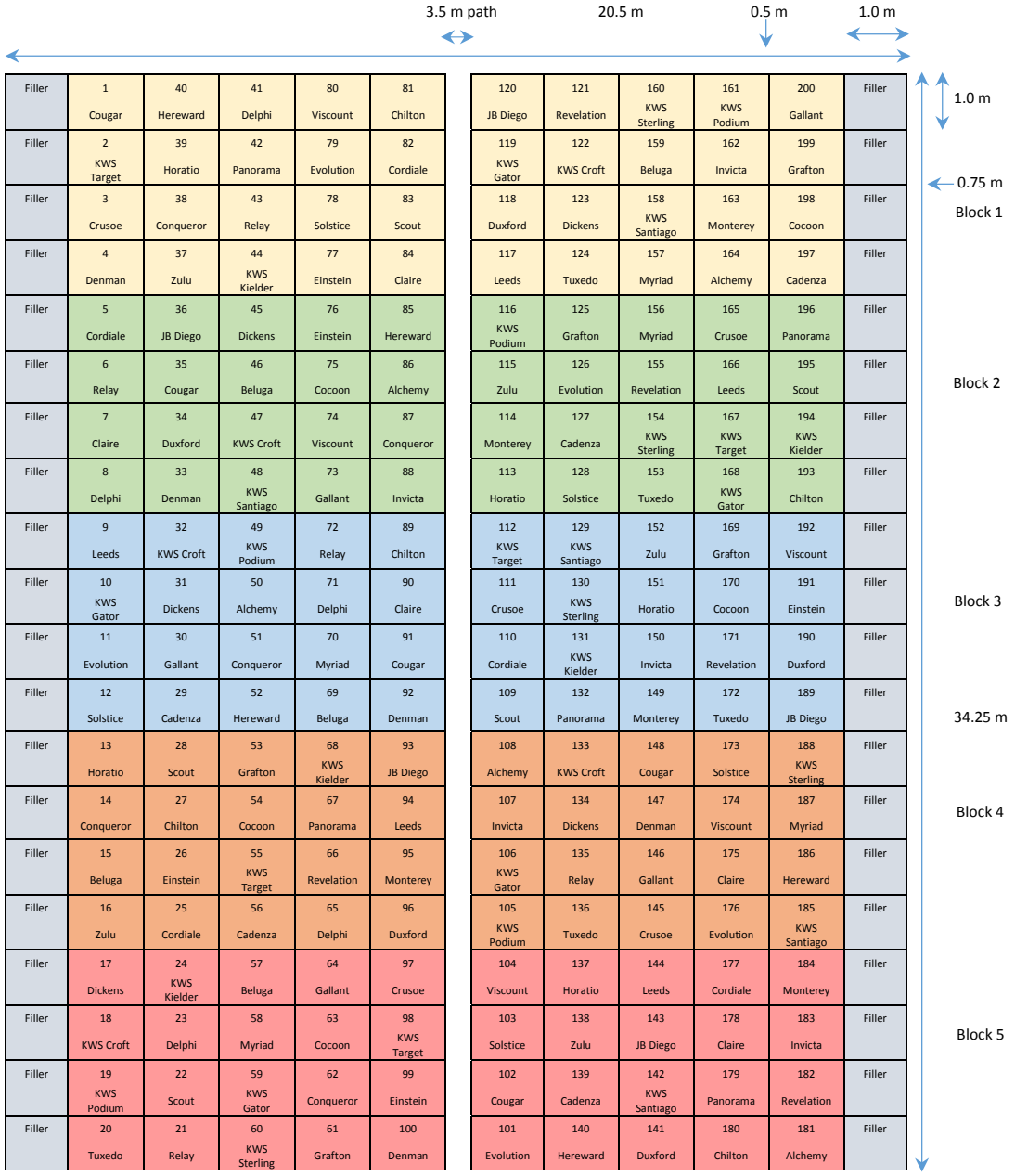
| | |
|---|---|
| CTAB | 10 g |
| TRISMA base | 6.06 g |
| EDTA | 1.46 g |
| NaCl | 20.5 g |
| | 500 ml |

**CIA:**

| | |
|---|---|
| Chloroform | 384 ml |
| Isoamyl alcohol | 16 ml |
| | 400 ml |

**Appendix 6.1. The ability of natural populations of *Phialophora* species to colonise the roots of the AHDB RL of winter wheat field trial plans.**

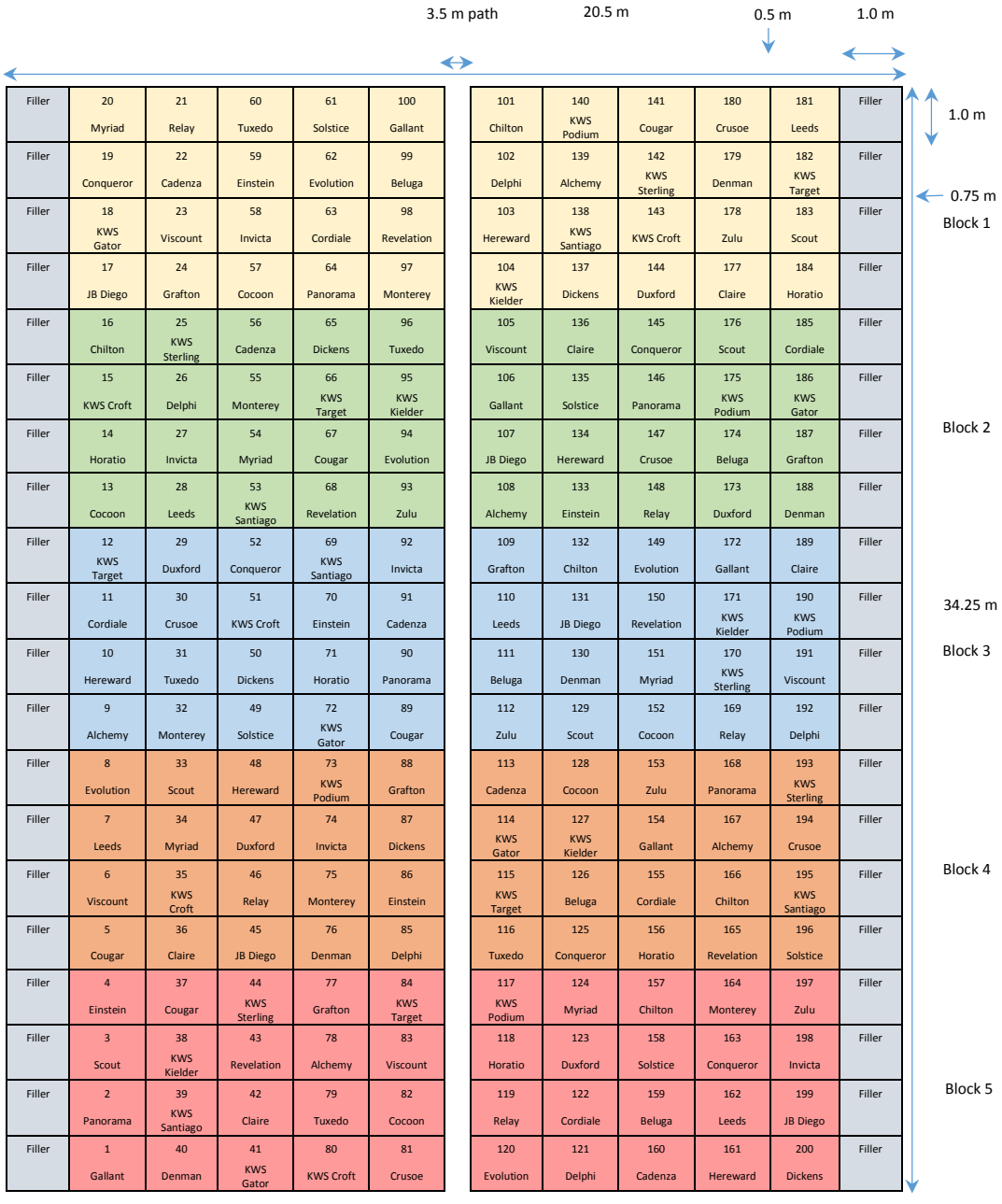
Field trial: 2015/R/WW/1516 Filler plots: JB Diego




| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Filler | 1 Cougar | 40 Hereward | 41 Delphi | 80 Viscount | 81 Chilton | | 120 JB Diego | 121 Revelation | 160 KWS Sterling | 161 KWS Podium | 200 Gallant | Filler |
| Filler | 2 KWS Target | 39 Horatio | 42 Panorama | 79 Evolution | 82 Cordiale | | 119 KWS Gator | 122 KWS Croft | 159 Beluga | 162 Invicta | 199 Grafton | Filler |
| Filler | 3 Crusoe | 38 Conqueror | 43 Relay | 78 Solstice | 83 Scout | | 118 Duxford | 123 Dickens | 158 KWS Santiago | 163 Monterey | 198 Cocoon | Filler |
| Filler | 4 Denman | 37 Zulu | 44 KWS Kielder | 77 Einstein | 84 Claire | | 117 Leeds | 124 Tuxedo | 157 Myriad | 164 Alchemy | 197 Cadenza | Filler |
| Filler | 5 Cordiale | 36 JB Diego | 45 Dickens | 76 Einstein | 85 Hereward | | 116 KWS Podium | 125 Grafton | 156 Myriad | 165 Crusoe | 196 Panorama | Filler |
| Filler | 6 Relay | 35 Cougar | 46 Beluga | 75 Cocoon | 86 Alchemy | | 115 Zulu | 126 Evolution | 155 Revelation | 166 Leeds | 195 Scout | Filler |
| Filler | 7 Claire | 34 Duxford | 47 KWS Croft | 74 Viscount | 87 Conqueror | | 114 Monterey | 127 Cadenza | 154 KWS Sterling | 167 KWS Target | 194 KWS Kielder | Filler |
| Filler | 8 Delphi | 33 Denman | 48 KWS Santiago | 73 Gallant | 88 Invicta | | 113 Horatio | 128 Solstice | 153 Tuxedo | 168 KWS Gator | 193 Chilton | Filler |
| Filler | 9 Leeds | 32 KWS Croft | 49 KWS Podium | 72 Relay | 89 Chilton | | 112 KWS Target | 129 KWS Santiago | 152 Zulu | 169 Grafton | 192 Viscount | Filler |
| Filler | 10 KWS Gator | 31 Dickens | 50 Alchemy | 71 Delphi | 90 Claire | | 111 Crusoe | 130 KWS Sterling | 151 Horatio | 170 Cocoon | 191 Einstein | Filler |
| Filler | 11 Evolution | 30 Gallant | 51 Conqueror | 70 Myriad | 91 Cougar | | 110 Cordiale | 131 KWS Kielder | 150 Invicta | 171 Revelation | 190 Duxford | Filler |
| Filler | 12 Solstice | 29 Cadenza | 52 Hereward | 69 Beluga | 92 Denman | | 109 Scout | 132 Panorama | 149 Monterey | 172 Tuxedo | 189 JB Diego | Filler |
| Filler | 13 Horatio | 28 Scout | 53 Grafton | 68 KWS Kielder | 93 JB Diego | | 108 Alchemy | 133 KWS Croft | 148 Cougar | 173 Solstice | 188 KWS Sterling | Filler |
| Filler | 14 Conqueror | 27 Chilton | 54 Cocoon | 67 Panorama | 94 Leeds | | 107 Invicta | 134 Dickens | 147 Denman | 174 Viscount | 187 Myriad | Filler |
| Filler | 15 Beluga | 26 Einstein | 55 KWS Target | 66 Revelation | 95 Monterey | | 106 KWS Gator | 135 Relay | 146 Gallant | 175 Claire | 186 Hereward | Filler |
| Filler | 16 Zulu | 25 Cordiale | 56 Cadenza | 65 Delphi | 96 Duxford | | 105 KWS Podium | 136 Tuxedo | 145 Crusoe | 176 Evolution | 185 KWS Santiago | Filler |
| Filler | 17 Dickens | 24 KWS Kielder | 57 Beluga | 64 Gallant | 97 Crusoe | | 104 Viscount | 137 Horatio | 144 Leeds | 177 Cordiale | 184 Monterey | Filler |
| Filler | 18 KWS Croft | 23 Delphi | 58 Myriad | 63 Cocoon | 98 KWS Target | | 103 Solstice | 138 Zulu | 143 JB Diego | 178 Claire | 183 Invicta | Filler |
| Filler | 19 KWS Podium | 22 Scout | 59 KWS Gator | 62 Conqueror | 99 Einstein | | 102 Cougar | 139 Cadenza | 142 KWS Santiago | 179 Panorama | 182 Revelation | Filler |
| Filler | 20 Tuxedo | 21 Relay | 60 KWS Sterling | 61 Grafton | 100 Denman | | 101 Evolution | 140 Hereward | 141 Duxford | 180 Chilton | 181 Alchemy | Filler |

**Appendix 6.1. Continued.**

Field trial: 2016/R/WW/1620 Filler plots: JB Diego




| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Filler | 20 Myriad | 21 Relay | 60 Tuxedo | 61 Solstice | 100 Gallant | 101 Chilton | 140 KWS Podium | 141 Cougar | 180 Crusoe | 181 Leeds | Filler |
| Filler | 19 Conqueror | 22 Cadenza | 59 Einstein | 62 Evolution | 99 Beluga | 102 Delphi | 139 Alchemy | 142 KWS Sterling | 179 Denman | 182 KWS Target | Filler |
| Filler | 18 KWS Gator | 23 Viscount | 58 Invicta | 63 Cordiale | 98 Revelation | 103 Hereward | 138 KWS Santiago | 143 KWS Croft | 178 Zulu | 183 Scout | Filler |
| Filler | 17 JB Diego | 24 Grafton | 57 Cocoon | 64 Panorama | 97 Monterey | 104 KWS Kielder | 137 Dickens | 144 Duxford | 177 Claire | 184 Horatio | Filler |
| Filler | 16 Chilton | 25 KWS Sterling | 56 Cadenza | 65 Dickens | 96 Tuxedo | 105 Viscount | 136 Claire | 145 Conqueror | 176 Scout | 185 Cordiale | Filler |
| Filler | 15 KWS Croft | 26 Delphi | 55 Monterey | 66 KWS Target | 95 KWS Kielder | 106 Gallant | 135 Solstice | 146 Panorama | 175 KWS Podium | 186 KWS Gator | Filler |
| Filler | 14 Horatio | 27 Invicta | 54 Myriad | 67 Cougar | 94 Evolution | 107 JB Diego | 134 Hereward | 147 Crusoe | 174 Beluga | 187 Grafton | Filler |
| Filler | 13 Cocoon | 28 Leeds | 53 KWS Santiago | 68 Revelation | 93 Zulu | 108 Alchemy | 133 Einstein | 148 Relay | 173 Duxford | 188 Denman | Filler |
| Filler | 12 KWS Target | 29 Duxford | 52 Conqueror | 69 KWS Santiago | 92 Invicta | 109 Grafton | 132 Chilton | 149 Evolution | 172 Gallant | 189 Claire | Filler |
| Filler | 11 Cordiale | 30 Crusoe | 51 KWS Croft | 70 Einstein | 91 Cadenza | 110 Leeds | 131 JB Diego | 150 Revelation | 171 KWS Kielder | 190 KWS Podium | Filler |
| Filler | 10 Hereward | 31 Tuxedo | 50 Dickens | 71 Horatio | 90 Panorama | 111 Beluga | 130 Denman | 151 Myriad | 170 KWS Sterling | 191 Viscount | Filler |
| Filler | 9 Alchemy | 32 Monterey | 49 Solstice | 72 KWS Gator | 89 Cougar | 112 Zulu | 129 Scout | 152 Cocoon | 169 Relay | 192 Delphi | Filler |
| Filler | 8 Evolution | 33 Scout | 48 Hereward | 73 KWS Podium | 88 Grafton | 113 Cadenza | 128 Cocoon | 153 Zulu | 168 Panorama | 193 KWS Sterling | Filler |
| Filler | 7 Leeds | 34 Myriad | 47 Duxford | 74 Invicta | 87 Dickens | 114 KWS Gator | 127 KWS Kielder | 154 Gallant | 167 Alchemy | 194 Crusoe | Filler |
| Filler | 6 Viscount | 35 KWS Croft | 46 Relay | 75 Monterey | 86 Einstein | 115 KWS Target | 126 Beluga | 155 Cordiale | 166 Chilton | 195 KWS Santiago | Filler |
| Filler | 5 Cougar | 36 Claire | 45 JB Diego | 76 Denman | 85 Delphi | 116 Tuxedo | 125 Conqueror | 156 Horatio | 165 Revelation | 196 Solstice | Filler |
| Filler | 4 Einstein | 37 Cougar | 44 KWS Sterling | 77 Grafton | 84 KWS Target | 117 KWS Podium | 124 Myriad | 157 Chilton | 164 Monterey | 197 Zulu | Filler |
| Filler | 3 Scout | 38 KWS Kielder | 43 Revelation | 78 Alchemy | 83 Viscount | 118 Horatio | 123 Duxford | 158 Solstice | 163 Conqueror | 198 Invicta | Filler |
| Filler | 2 Panorama | 39 KWS Santiago | 42 Claire | 79 Tuxedo | 82 Cocoon | 119 Relay | 122 Cordiale | 159 Beluga | 162 Leeds | 199 JB Diego | Filler |
| Filler | 1 Gallant | 40 Denman | 41 KWS Gator | 80 KWS Croft | 81 Crusoe | 120 Evolution | 121 Delphi | 160 Cadenza | 161 Hereward | 200 Dickens | Filler |

**Appendix 6.2. Fungal isolate ID's and NCBI accession numbers of isolates in the phylogenetic analysis.**

| Isolate ID used in phylogenetic analysis | Accession number in NCBI |
|---|---|
| **BLAST hits of field isolates from 2015/R/WW/1516** | |
| *Gaeumannomyces graminis* strain V2FR3 | KT692582.1 |
| *Gaeumannomyces graminis* strain SMCD | JQ658339.1 |
| *Gaeumannomyces graminis* var. *graminis* isolate 85/23-1 | AJ010033.1 |
| *Gaeumannomyces radicicola* strain ATCC | KM484850.1 |
| *Phialophora* sp. lobed hyphopodia P2/P9 | U17216.1 |
| ***Gaeumannomyces graminis* var. *tritici* reference sequences** | |
| *Gaeumannomyces graminis* var. *tritici* 1 | AF508156.1 |
| *Gaeumannomyces graminis* var. *tritici* 2 | AF508155.1 |
| *Gaeumannomyces graminis* var. *tritici* 3 | AF511489.1 |
| *Gaeumannomyces graminis* var. *tritici* 4 | AF087684.1 |
| *Gaeumannomyces graminis* var. *tritici* strain 9735 | AF508789.1 |
| *Gaeumannomyces graminis* var. *tritici* strain 9840 | AF508788.1 |
| *Gaeumannomyces graminis* var. *tritici* isolate 87/7-4 | AJ010037.1 |
| *Gaeumannomyces graminis* var. *tritici* isolate 90/2-2 | AJ010036.1 |
| *Gaeumannomyces graminis* var. *tritici* isolate ADB15 | FJ541432.1 |
| *Gaeumannomyces graminis* var. *tritici* isolate ARS-A1 | FJ541431.1 |
| *Gaeumannomyces graminis* var. *tritici* AT2 | U17209.1 |
| *Gaeumannomyces graminis* var. *tritici* AT3 | U17210.1 |
| *Gaeumannomyces graminis* var. *tritici* strain CBS 186.65 | KM484838.1 |
| *Gaeumannomyces graminis* var. *tritici* strain CBS 247.29 | KM484839.1 |
| *Gaeumannomyces graminis* var. *tritici* strain CBS 249.29 | KM484840.1 |
| *Gaeumannomyces graminis* var. *tritici* strain CBS 905.73 | KM484841.1 |
| *Gaeumannomyces graminis* var. *tritici* strain GaNS-90 | KF150197.1 |
| *Gaeumannomyces graminis* var. *tritici* strain GaYJ-5 | KF150198.1 |
| *Gaeumannomyces graminis* var. *tritici* GGT19 | AY428783.1 |
| *Gaeumannomyces graminis* var. *tritici* strain GGTOR2 | AY428784.1 |
| *Gaeumannomyces graminis* var. *tritici* isolate M55 | JF414850.1 |
| *Gaeumannomyces graminis* var. *tritici* isolate PD19 | FJ541433.1 |
| *Gaeumannomyces graminis* var. *tritici* R1 | U17219.1 |
| *Gaeumannomyces graminis* var. *tritici* R11 and R17 | U17220.1 |
| *Gaeumannomyces graminis* var. *tritici* isolate R11-3-3D | AJ969262.1 |
| *Gaeumannomyces graminis* var. *tritici* isolate R3-111a-1 | FJ541430.1 |
| *Gaeumannomyces graminis* var. *tritici* haplotype S1 | FJ771001.1 |
| *Gaeumannomyces graminis* var. *tritici* haplotype S2 | FJ771002.1 |
| *Gaeumannomyces graminis* var. *tritici* haplotype S3 | FJ771003.1 |
| *Gaeumannomyces graminis* var. *tritici* haplotype S4 | FJ771004.1 |
| *Gaeumannomyces graminis* var. *tritici* haplotype S5 | FJ771005.1 |
| *Gaeumannomyces graminis* var. *tritici* T2 | U17221.1 |
| *Gaeumannomyces graminis* var. *tritici* T5 | U17222.1 |

**Appendix 6.2. Continued.**

| Isolate ID used in phylogenetic analysis | Accession number in NCBI |
|---|---|
| ***Gaeumannomyces graminis* var. *tritici* reference sequences** | |
| *Gaeumannomyces graminis* var. *tritici* isolate TN Ggt 15 | KT819300.1 |
| *Gaeumannomyces graminis* var. *tritici* isolate TN Ggt 2 | KT819302.1 |
| ***Phialophora* spp. reference sequences** | |
| *Gaeumannomyces graminis* var. *graminis* isolate 247 | AJ010034.1 |
| *Gaeumannomyces graminis* var. *graminis* isolate W4066A | AJ010032.1 |
| *Phialophora graminicola* P4 | U17217.1 |
| *Phialophora graminicola* P7 and P8 | U17218.1 |
| *Phialophora* sp. isolate GP57 | AJ010039.1 |
| *Phialophora* sp. 437 | AJ132542.1 |
| *Phialophora* sp. K8 | AJ132541.1 |
| ***Magnaporthe grisea outlier group*** | |
| *Magnaporthe grisea* BR0029 | KM484880.1 |
| *Magnaporthe grisea* CR0024 | KM484882.1 |

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