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

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CONTENTS

1. 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.

2. Introduction

2.1. 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).

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. 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 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, that have been found to disrupt water and nutrient uptake of the plant (Pillinger et al., 2005).

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 usually

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 (Freeman and Ward, 2004). 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.

2.2. Take-all control methods

2.2.1. Cultural control methods

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 a subsequent susceptible crop. This is due to the poor saprotrophic survival of the fungus in the absence of a host crop as *Ggt* becomes uneven amongst the soil and begins to decompose (Hornby et al., 1998). Other cultural control methods consist of a delayed sowing date to allow the *Ggt* inoculum to decline in the soil in the intercrop period and correcting soil nutrient deficiencies, such as phosphate, potassium, manganese and sulphur, prior to drilling the crop.

2.2.2. Chemical control methods

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). However, 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. 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). Yet, it has been reported that silthiofam efficacy on take-all has been declining in farmers' 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 (Gutteridge and Hammond-Kosack, 2008).

2.2.3. Biological control methods

2.2.3.1 Take-all Decline

A number of microorganisms have been found to suppress take-all disease, including bacterial species involved in the natural phenomenon of Take-all Decline (TAD). TAD is a natural 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). 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). TAD is exploited by farmers, knowingly or not, through the agricultural practice of continuous cereals (Cook, 2003). TAD 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 are and studies have suggested that a different microbial composition is responsible for TAD in different geographical regions (de Souza et al., 2003).

2.2.3.2. *Phialophora*

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). 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).

The two main *Phialophora* species in the *Gaeumannomyces-Phialophora* complex found in agricultural soils are *Phialophora* sp. lobed hyphopodia and *Phialophora graminicola*. Both fungi are able to colonise a range of grasses. *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).

Phialophora sp. lobed hyphopodia is pathogenic on rice and Bermuda grass but weakly/non-pathogenic on cereals including wheat (Freeman and Ward, 2004). *Phialophora graminicola* 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, 1973). 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). 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.

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 *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).

A diverse array of molecular diagnostic methods is also available to differentiate species within the *Gaeumannomyces-Phialophora* complex but few elicit reliable specificity. 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 project 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.

A recent taxonomical reclassification of the Magnaporthaceae family has been carried out by Hernández-Restrepo et al. (2016) and a number of name changes have been given to species within the *Gaeumannomyces-Phialophora* complex. This study refers to the *Gaeumannomyces-Phialophora* nomenclature but future literature will refer to the new taxonomical classifications, which can be found in the Hernández-Restrepo et al. (2016) study.

2.2.4. Take-all resistant wheat germplasm

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 or inconsistent to be of beneficial use (Hornby et al., 1998). Studies have been conducted in Australia that have identified a moderate 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 take-all than Bayonet. More recent work based at Rothamsted Research, within the Wheat Genetic Improvement Network (WGIN) and the Wheat Improvement Strategic Programme (WISP), has screened 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, for resistance against take-all disease in the field. Moderate resistance has been identified in a number of varieties (WGIN Stakeholder's Newsletter, April 2014). 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.

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. 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 (highly resistant) and triticale (moderately resistant) in their level of susceptibility (McMillan et al., 2014). A mapping population between the take-all susceptible *T. monococcum* accession MDR037 and the moderately take-all resistant *T. monococcum* accession MDR046 was screened in third wheat field trials within this PhD project.

2.3. 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 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.

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.

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.

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

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

Hypothesis: the ability of *Phialophora* fungi to colonise the RL winter wheat varieties will vary.

3. Materials and methods

3.1. First and third wheat field trials

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* fungi under current elite wheat varieties. 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. All field trial designs were generated by statistician Rodger White using a randomised block design in CycDesigN (VSN International Ltd, Hemel Hempstead, UK). Field trials were established on the Rothamsted Farm, on flinty clay soil of the Batcombe soil series. 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. In addition, no growth regulators and only one dose of the herbicide Pacifica (mesosulfuron-methyl + iodosulfuron-methyl-sodium) were applied to the diploid *Triticum monococcum* wheat plots. Diploid *T. monococcum* wheat plots were weeded by hand where the Pacifica was not sufficient to control weeds throughout the whole field season.

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 was used to score each plot using a scale of 0-10, 0 representing no senescence and 10 representing fully senesced flag leaves. 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.

Plant samples were taken during each summer, from the medium milk development stage onwards (growth stage 75), to assess for take-all root infection in the third wheat field trials and *Phialophora* colonisation in the first wheat field trial. String 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. 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.

The dried plant root systems were soaked in water for approximately 20 minutes before disease assessments. Any remaining soil was then washed off and the root systems were immersed in water in a white dish. Within the third wheat field trials, 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. *Phialophora* assessments are described in section 3.1.3.

3.1.1. Third wheat field trials screening the 25 most resistant hexaploid varieties

Two third wheat field trials were used 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. 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 in the third wheat field trials screening the MDR037 X MDR046 mapping population. 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*). Take-all is a notoriously patchy disease and to enable the spatial variation of the disease to be accounted for during the statistical analysis, 40 replicates of the winter wheat variety Hereward (highly susceptible to *Ggt*) were also included. Lines were hand-harvested from the field trial to provide seed for the 2014-2015 replicated field trial. The seed rate per plot was 350 seeds/m^2 and plot size was 6 rows of 1 m x 1 m.

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 in the two field trials. 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. During the 2013-2014

field trial, one of the Hereford plots was not sown in the field trial due to a problem when drilling the trial, a soil moisture sensor was inserted into this plot and used as a fallow control. During the 2014-2015 field trial, two soil moisture sensors were inserted into bare soil to the north and south of the field trial as two fallow controls. Once the field 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 15 cm until the four stainless steel rods were set firmly into the soil. 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. Sensors were removed after plant sampling.

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:

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

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) (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). 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. 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 degree of saturation for each plot of each variety was 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. Above-ground phenotypic crop measurements and plant samples were taken as described in section 3.1.

3.1.2. Third wheat field trials screening the diploid *Triticum monococcum* mapping population MDR037 (Susceptible) X MDR046 (Resistant)

Two third wheat field trials were used to screen the diploid *Triticum monococcum* mapping population MDR037 (S) X MDR046 (R) in the 2013-2014 and 2014-2015 field seasons for resistance to take-all disease. In the 2013-2014 field trial, the trial consisted of five replicates of the 72 $F_{5:}F_6$ *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population lines, five replicates of the two *T. monococcum* parental lines and 40 replicates of the hexaploid variety Hereward as a highly susceptible control to take-all. 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 2014-2015 repeat field trial. The 2014-2015 trial therefore consisted of five replicates of 79 F_5:F_6/F_5:F_7 *T. monococcum* lines, five replicates of the two T. monococcum parental lines and 41 replicates of the hexaploid control variety Hereward. Seed rates per plot were 80 seeds and plots consisted of 4 rows (0.6 m length). Above-ground phenotypic crop measurements and plant samples were taken as described in section 3.1.

3.1.3. First wheat field trial screening the AHDB Recommended List of winter wheat varieties for their ability to build-up natural populations of *Phialophora* fungi

The 2013/2014 AHDB RL for winter wheat was screened in a first wheat trial to explore the ability of *Phialophora* fungi to colonise the elite wheat varieties as well as the ability of *Phialophora* fungi to build-up under a first wheat crop. Plant samples and above-ground phenotypic crop measurements were taken as described in section 3.1. Yields were taken from the trial by the Rothamsted Farm.

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, 3 mm 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 processing over the following months. During processing the soil cores were sprayed with water and ten seeds of each variety were placed on top of the soil, three cores per plot were baited with Hereward and three cores per plot were baited with the field plot variety. 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 (Figure 1). 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.

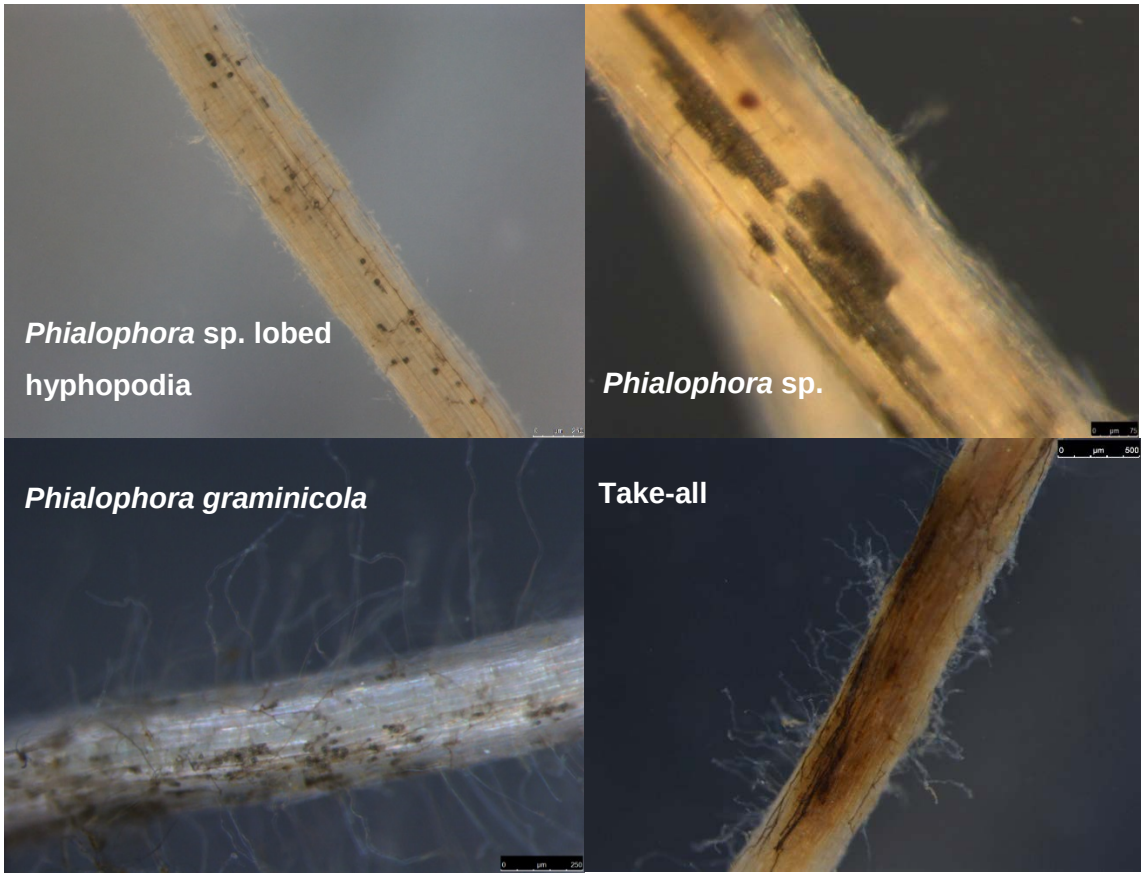

Figure 1. Microscopy images taken during this project of wheat seedling roots colonised by *Phialophora* sp. lobed hyphopodia, the unnamed *Phialophora* sp., *Phialophora graminicola* and infected by take-all.

To evaluate the ability of *Phialophora* fungi to colonise the adult plants of the 2013/2014 RL, a subset of plant samples from ten plots, chosen due to high levels of *Phialophora* colonisation found within the assessed post-harvest soil core bioassays, were visually assessed for *Phialophora* colonisation. No sub-epidermal vesicles could be seen by eye within the adult root systems, or under the light microscope, and the remaining samples were not assessed.

3.2. Gathering a *Phialophora* isolation collection

Five fields were sampled across the Rothamsted Farm during 2013 and one field during 2014. 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 sub-epidermal vesicles of *Phialophora* colonisation. *Phialophora* spp. were then isolated from colonised root pieces by surface sterilising the roots in sodium hypochlorite for 5 minutes before triple washing in sterilised H_2O, blotting dry on sterilised filter paper and plating onto potato dextrose agar (PDA) amended with penicillin (50 µg per PDA plate) and streptomycin (50 µg per PDA plate) in a sterilised air flow hood. Plates were incubated at 21°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.

A 1 cm^3 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. 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. 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. 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.

PCR was then performed 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.

3.3. Development of a *Phialophora* pot bioassay to screen cereal germplasm

In June 2014 a 10 week AHDB summer bursary project, with undergraduate student Brittany Burton, evaluated the susceptibility of cereal genotypes to a *Phialophora* sp. lobed hyphopodia isolate and a *Phialophora* sp. isolate. 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 have previously been screened for take-all susceptibility, Paragon is susceptible to take-all and Watkins line 777 is partially resistant. The *T. monococcum* accessions MDR037 (S) and MDR046 (R) were the parental lines of the mapping population screened in the third wheat field trials. 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).

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 mm diameter) were filled with a 50 cm^3 layer of damp coarse sand. The PDA plate inoculum 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. 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 were then statistically analysed using ANOVA.

3.4. Generation of the draft genomes of three *Phialophora* species

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 1^{st} wheat field trial to screen the ability of *Phialophora* species to build-up under elite winter wheats, New Zealand field on the RRes Farm (Table 1). 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, amended with penicillin (50 µg) and streptomycin (50 µg), in a sterile air flow hood. The plates were incubated at 15°C until confluent. A 1 cm^3 agar plug was then cut from the confluent plates of each of the three *Phialophora* isolates 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 from 2 ml ground freeze-dried mycelium with 15/20 ml CTAB (cetyltrimethylammonium bromide) lysis buffer. 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) 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. An additional phenyl/chloroform extraction was carried out on the *Phialophora* sp. lobed hyphopodia and *Phialophora graminicola* isolate submissions in January 2015 and *Phialophora* sp. lobed hyphopodia isolate submissions in May 2015 (Table 2). DNA was purified using Qiagen Plasmid or Cosmid DNA Purification Kit and following the Plasmid Midi Kit protocol from step 9. 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.

The extracted *Phialophora* spp. DNA was sent to the Earlham Institute (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 was successfully sequenced in the third and final batch (Table 2). Illumina sequencing was performed by the EI. The Illumina HiSeq 2500 sequencing platform was used to generate 125 bp paired-end reads.

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.*

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. 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).

Table 1. 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] Isolate described in Ward and Gray (1992).

Table 2. 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[1,2]	*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.

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.

3.5. Statistics

Statistical analyses were performed in the software Genstat (VSN International Ltd, Hemel Hempstead, UK). The field trial data for the percentage take-all disease data and *Phialophora* colonisation data were transformed using the logit transformation and were statistically analysed by statistician Rodger White. The above-ground crop measurements for the each of the field trials were also statistically analysed. 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.

4. Results

4.1. Third wheat field trials screening the diploid *Triticum monococcum* mapping population MDR037 (Susceptible) X MDR046 (Resistant)

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) (Figure 2). 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 (Figure 2). 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 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 due to winter flooding across part of the site. No trend was found for the level of plant establishment of the plot on the TAI score. 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 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.

The 2014-2015 field trial screened an additional seven MD0R37 (S) X MDR046 (R) mapping population lines (n = 79 lines) and significant differences were detected for the control varieties and mapping population lines in the TAI (P = 0.001) (Figure 3). 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) (Figure 2 & Figure 3). 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 and a TAI of 40.62 for the susceptible parental line MDR037 (Figure 3). 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. *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).

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 (Figure 4, *P* = <0.001). The SED for the combined year analysis is smaller (Figure 4) than the SED for the each of the individual years (Figure 2 & Figure 3), 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 (Figure 4). 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 9th 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.

Above-ground phenotypic crop measurements for the 2013-2014 field trial indicated that there were significant effects of genotype on days to anthesis (d.f.= 74, SED= 1.379, *P*= <0.001), days to leaf senescence (d.f.= 74, SED= 1.551, *P*= <0.001) and plant height (d.f.= 74, SED= 5.172, *P*= <0.001). 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. 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. 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 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). The earlier that the leaves senesced, the higher the take-all index. 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).

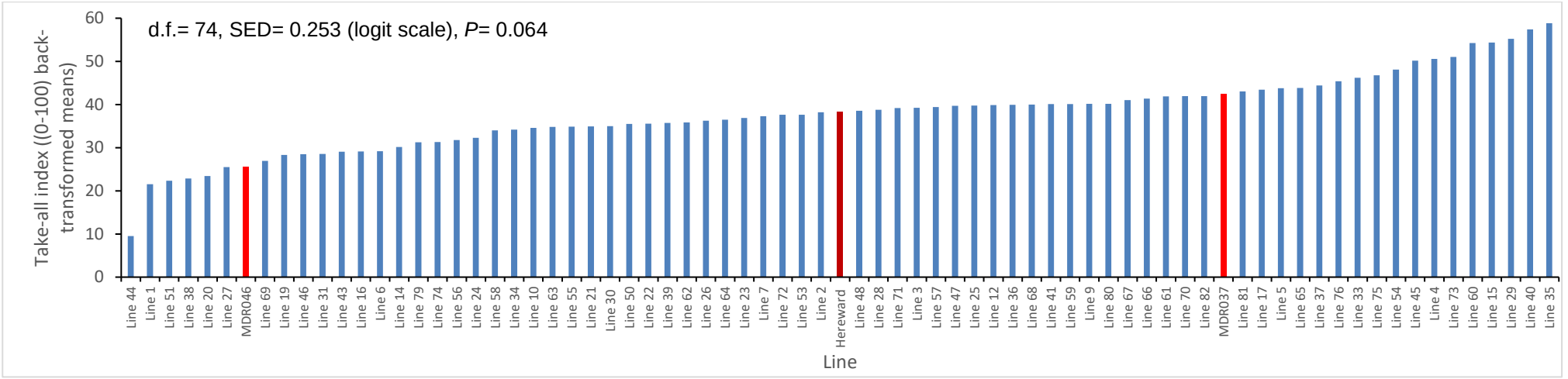

Figure 2. Take-all disease across the *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population in a third wheat field trial for the **2013-2014** field season.

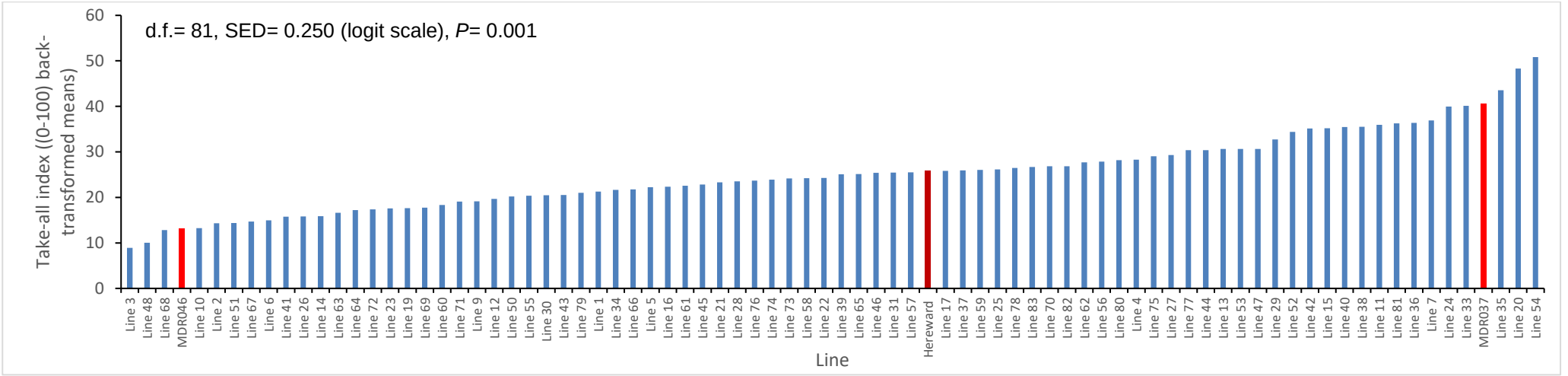

Figure 3. Take-all disease across the *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population in a third wheat field trial for the **2014-2015** field season.

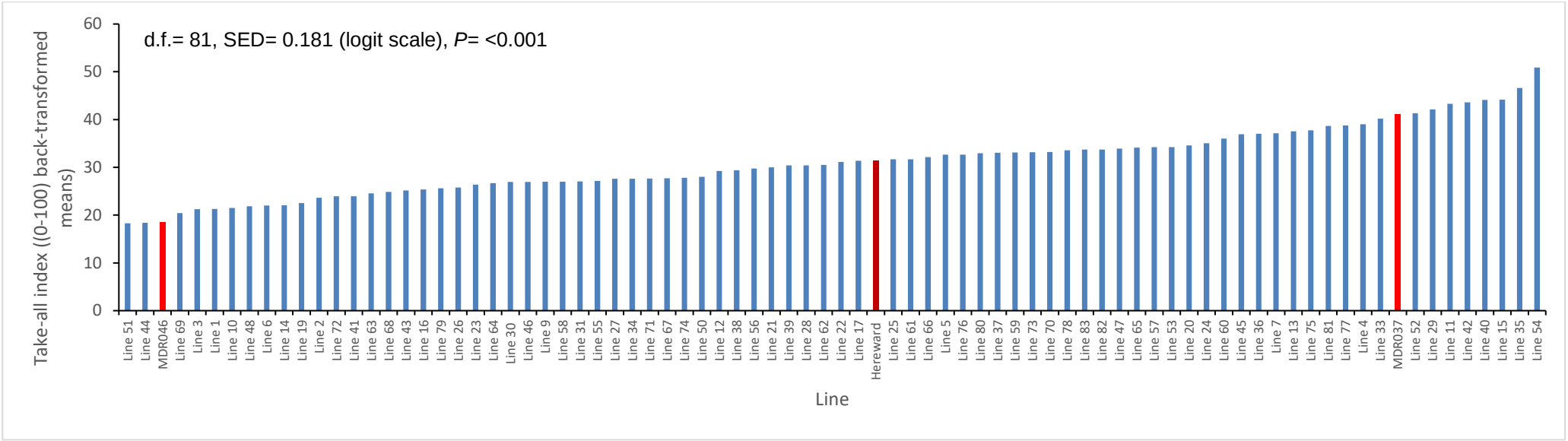

Figure 4. Combined year analysis of take-all disease across the *Triticum monococcum* MDR037 (S) X MDR046 (R) mapping population in third wheat field trials for the 2013-2014 and 2014-2015 field seasons.

The above-ground phenotypic crop measurements for the 2014-2015 field trial also indicated that there were significant effects of genotype on days to anthesis (d.f.= 81, SED= 0.876, *P*= <0.001), days to leaf senescence (d.f.= 81, SED= 1.197, *P*= <0.001) and plant height (d.f.= 81, SED= 3.044, *P*= <0.001). 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: 9th June; 2014: 13th 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 than 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. 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).

4.2. Third wheat field trials screening the 25 most resistant hexaploid varieties

The 2013-2014 field trial screened 33 cereal varieties and a significant difference for the TAI was found across the varieties (*P* = <0.001) (Figure 5). The TAI for the control varieties (rye, triticale and Hereward) ranged from 6.86 - 53.88 (Figure 5). 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 moderate level of resistance to take-all, namely 487, 500 and 739. 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.

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 in the 2014-2015 field trial (Figure 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) (Figure 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 2013-2014 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).

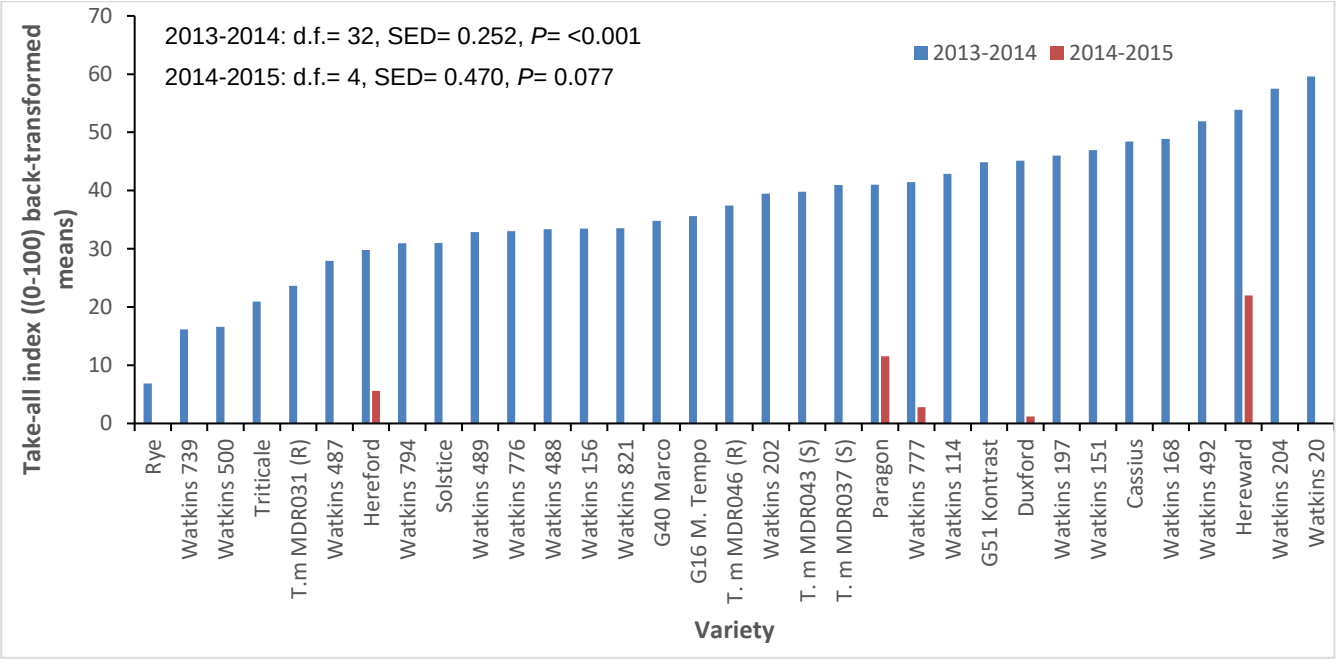

Figure 5. Take-all disease susceptibility of the 25 most resistant take-all hexaploid varieties in a third wheat field trial in the 2013-2014 field season and a subset of 5 varieties in the 2014-2015 field season.

The degree of saturation of each plot replicate was 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. As shown previously in Figure 5, the three moderately resistant varieties had no less disease than the susceptible varieties in this field trial.

In the 2013-2014 field trial, 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 6). 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, yet less of a relationship was found between the degree of saturation and the TAI for the take-all susceptible variety Paragon (Figure 6). 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 6). 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 6).

Overall, the 2014-2015 field trial had a lower level of take-all pressure than seen in the 2013-2014 field trial (Figure 5). The ranking of the TAI score varied across the blocks for each variety (Figure 7). 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 7). 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 7). However, there appeared to be no relationship between the degree of saturation and the TAI across the plot replicates for either variety (Figure 7). This could be due to the low overall take-all index and that it is presumed that a certain threshold of take-all would be required to have an effect on soil moisture.

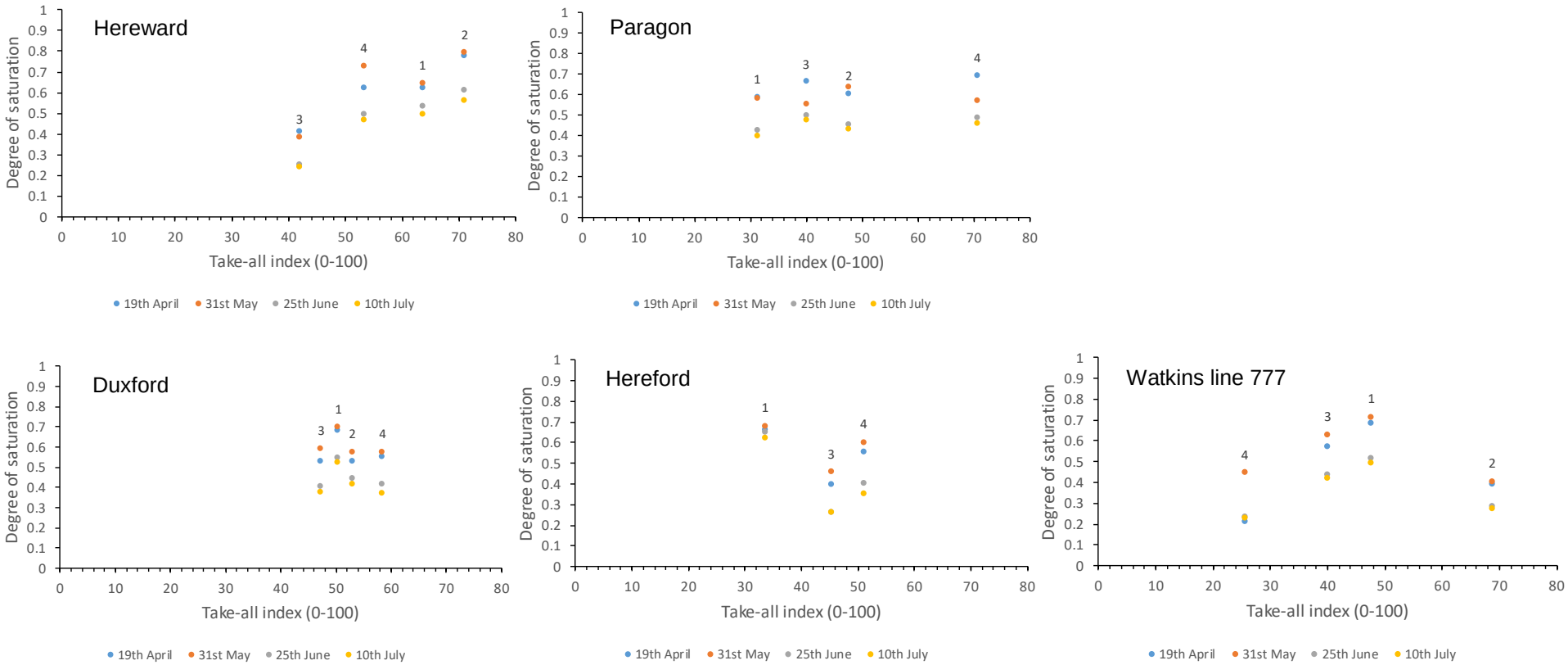

Figure 6. Degree of saturation for three or four plot replicates (experimental blocks numbered above) of the take-all susceptible varieties Hereward and Paragon and the moderately resistant varieties Duxford, Hereford and Watkins line 777 plotted against the take-all index (TAI) for 19th April, 31st May, 25th June and 10th July of the **2013-2014** (2014/R/WW/1419) field trial.

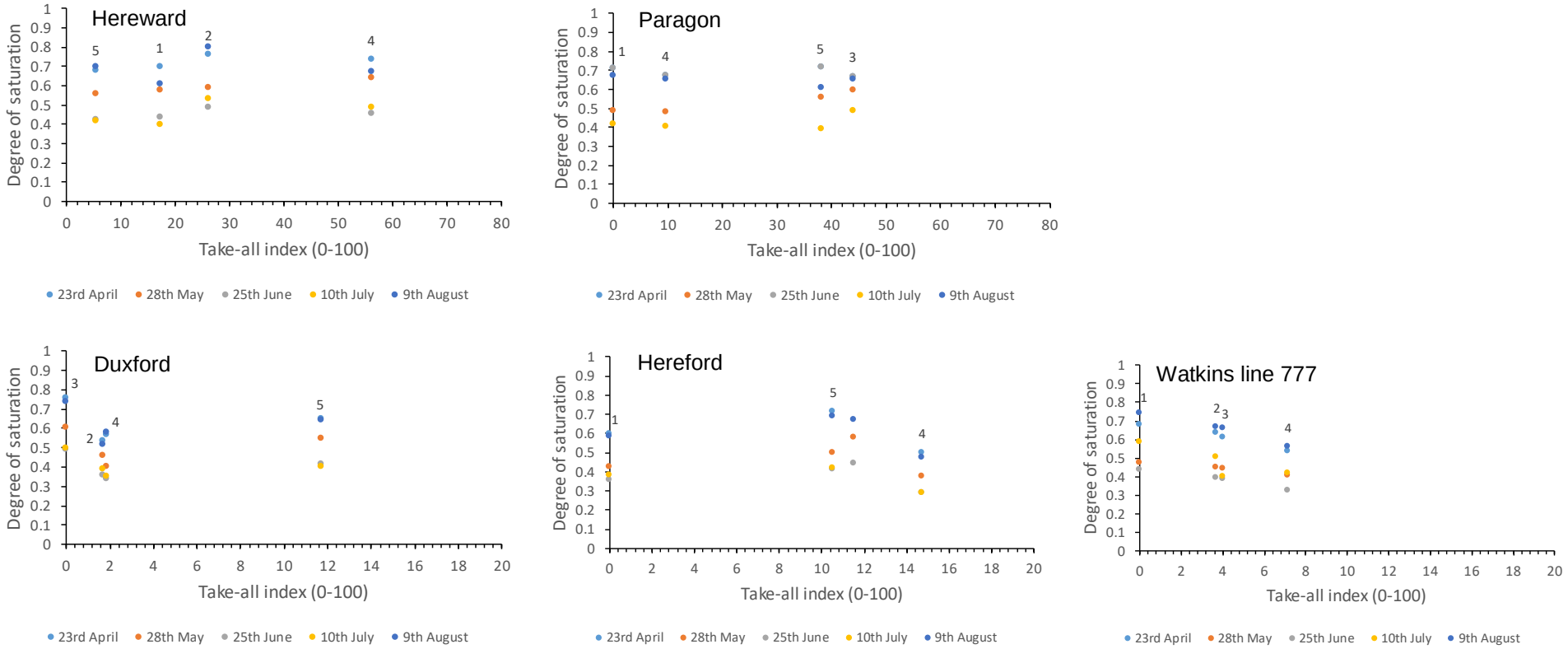

Figure 7. Degree of saturation for four plot replicates (experimental blocks numbered above) of the take-all susceptible varieties Hereward and Paragon and the moderately resistant varieties Duxford, Hereford and Watkins line 777 plotted against the take-all index (TAI) for 23rd April, 28th May, 25th June, 10th July and 9th August of the **2014-2015** (2015/R/WW/1510) field trial.

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. During the 2013-2014 field season, there was a major rainfall event on 27th May 2014, with a total daily rainfall of 11.5 mm, and the next six consecutive days were plotted against the TAI for each individual plot (Figure 8). 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 8). No trend in the level of soil drying was found across the four Paragon plot replicates (Figure 8). 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 8). 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 8). 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 8). 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.

During the 2014-2015 field season, there was an extreme rainfall event on 16th July 2015 where a total daily rainfall of 57.1 mm fell. The next six consecutive days were plotted against the TAI for each individual plot (Figure 9). 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 9). The majority of the remaining plot replicates of the five varieties, illustrated a substantial drop in the degree of saturation from the 17th July to the 18th July followed by slight soil drying (Figure 9). 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.

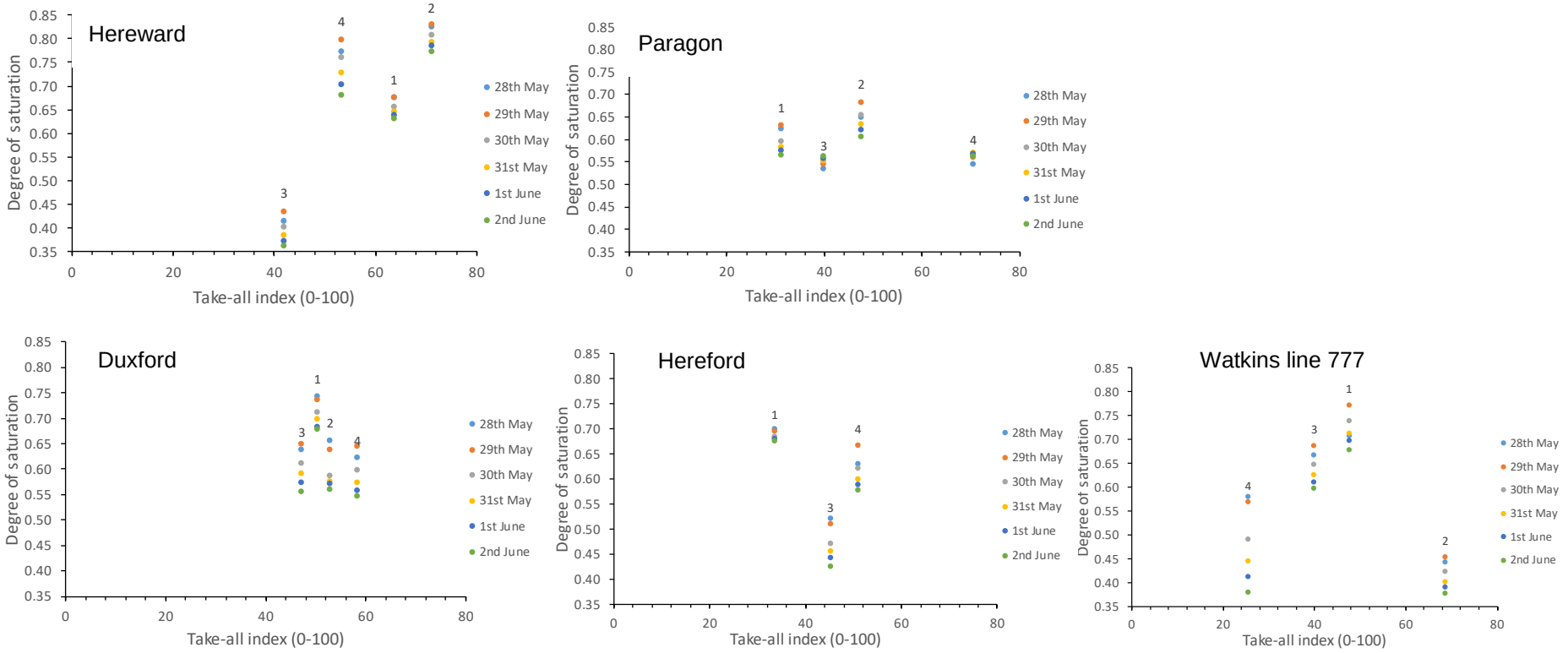

Figure 8. The level of soil drying after a major rainfall event on the 27th May 2014. The degree of saturation for the three or four plot replicates (experimental blocks numbered above) of the take-all susceptible varieties Hereward and Paragon and the moderately resistant varieties Duxford, Hereford and Watkins line 777 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.

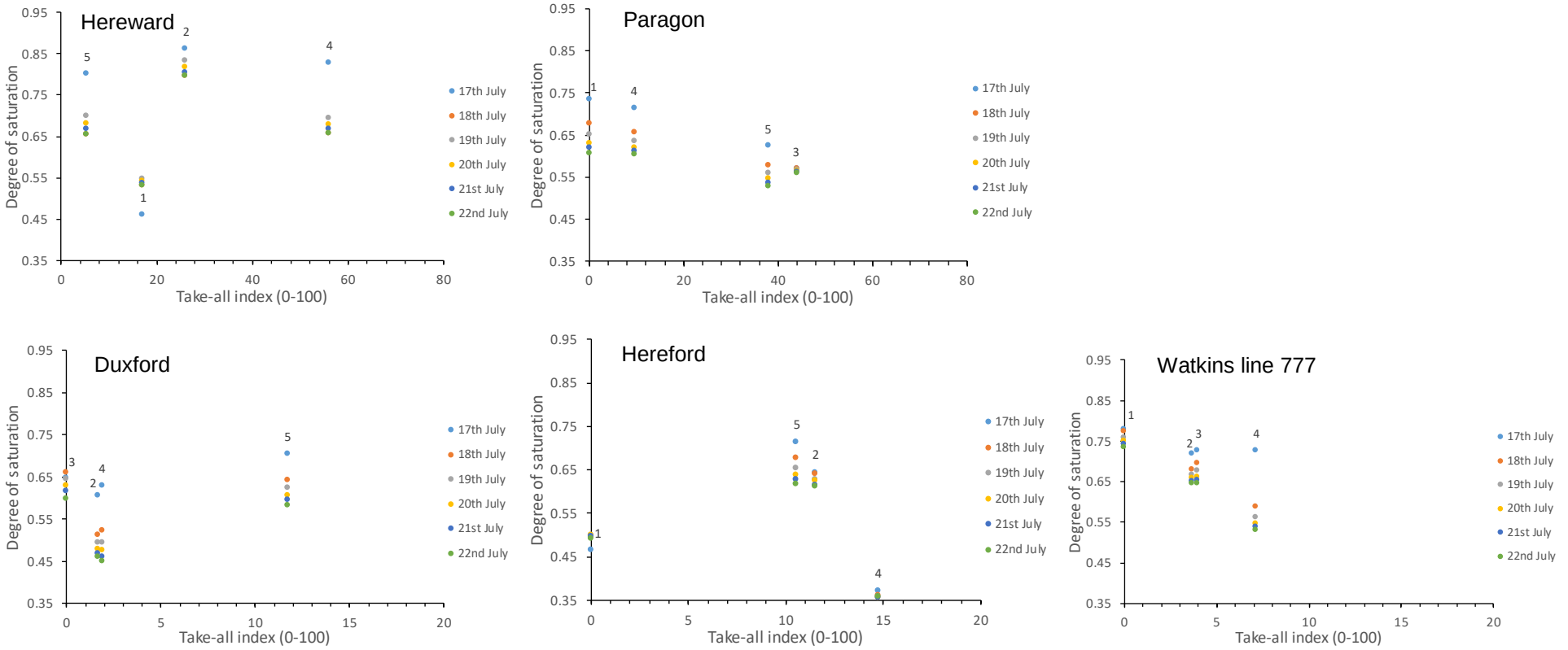

Figure 9. The level of soil drying after a major rainfall event on the 16th July 2015. The degree of saturation for four plot replicates (experimental blocks numbered above) of the take-all susceptible varieties Hereward and Paragon and the moderately resistant varieties Duxford, Hereford and Watkins line 777 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.

4.3. Gathering a *Phialophora* isolate collection

Nine field isolates were visually identified as *Phialophora* species due to the presence of sub-epidermal vesicles. The nine isolates were then confirmed as eight isolates of *Phialophora* sp. lobed hyphopodia (Figure 10) and one isolate of an unnamed *Phialophora* sp. (Figure 11) (Table 3) through ITS sequencing. The unnamed *Phialophora* sp. has previously been isolated at RRes (Ward and Bateman, 1999) and in Germany (Ulrich et al., 2000). Unfortunately, a *Phialophora graminicola* isolate could not be isolated from the field and a cold room stock of a *P. graminicola* isolate, that was first isolated at RRes in 1974 (Ward and Gray, 1992), was subcultured to provide a complete isolate collection of the three *Phialophora* species currently found in UK soils.

Table 3. 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] Isolate description in Ward and Gray (1992).

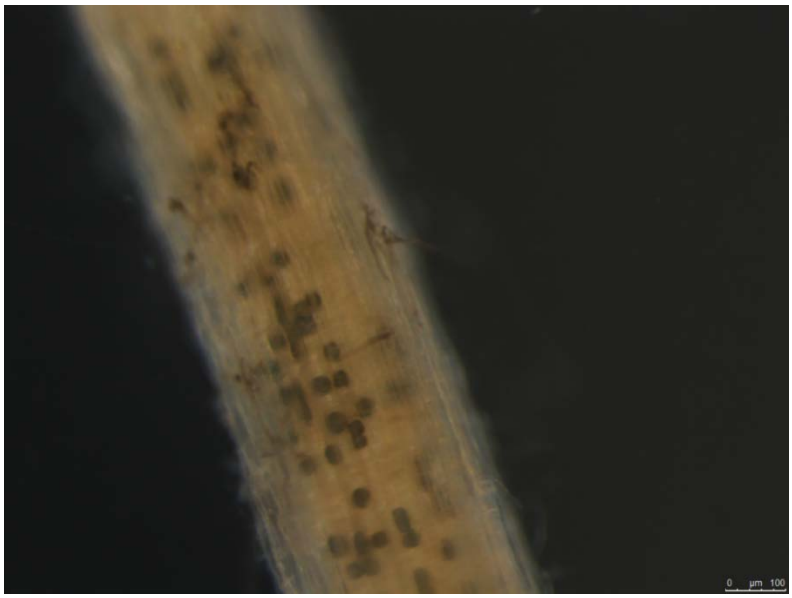

Figure 10. Microscopy image of *Phialophora* sp. lobed hyphopodia, field isolate P.10.13, colonised wheat seedling root. Magnification X 111.

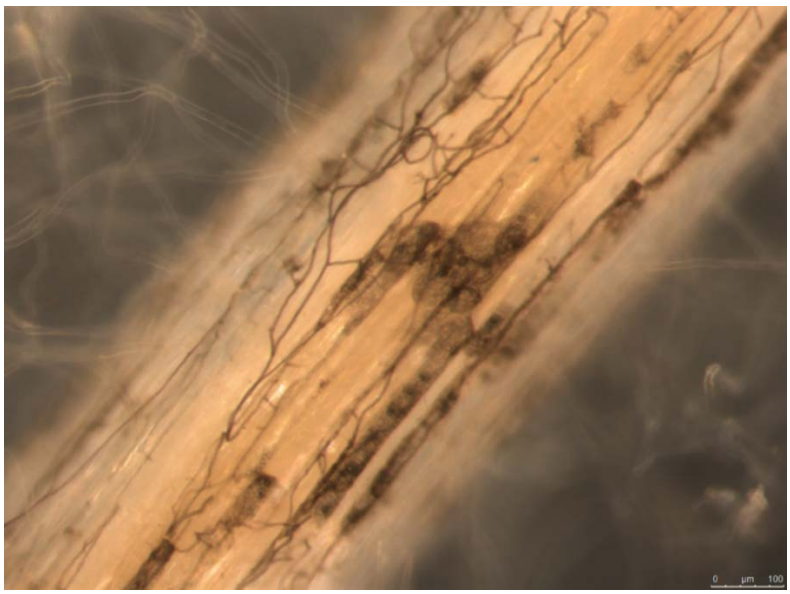

Figure 11. Microscopy image of *Phialophora* sp., field isolate S.09.13, colonised wheat seedling root. Magnification X 134.

4.4. Development of a *Phialophora* pot bioassay to screen cereal germplasm

An experimental pot bioassay was carried out by AHDB summer bursary student Brittany Burton to screen the ability of *Phialophora* spp. to colonise the roots of selected cereal genotypes. 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) (Figure 12). 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 (Figure 12). 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: d.f.= 8, SED = 0.568, *P* = <0.001). *Phialophora*-like colonisation was found on either no roots or 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. Unfortunately, the isolate was not recovered. A significant difference in the total number of roots per plant across the cereal genotypes was found for *Phialophora* spp. (ANOVA: d.f. = 8, SED = 0.376, *P* = <0.001), with rye and triticale having the highest number of roots and Hereward and the *T. monococcum* accession MDR046 having the lowest number of roots. 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: d.f. = 1, SED = 0.177, *P* = 0.034). 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 (Figure 12). A slightly lower grand mean of *Phialophora* sp. lobed colonised roots was found compared to the first PDA pot bioassay but again, the percentage of roots colonised by *Phialophora sp.* was higher than *Phialophora* sp. lobed hyphopodia for all cereal genotypes, except in rye (Figure 12).

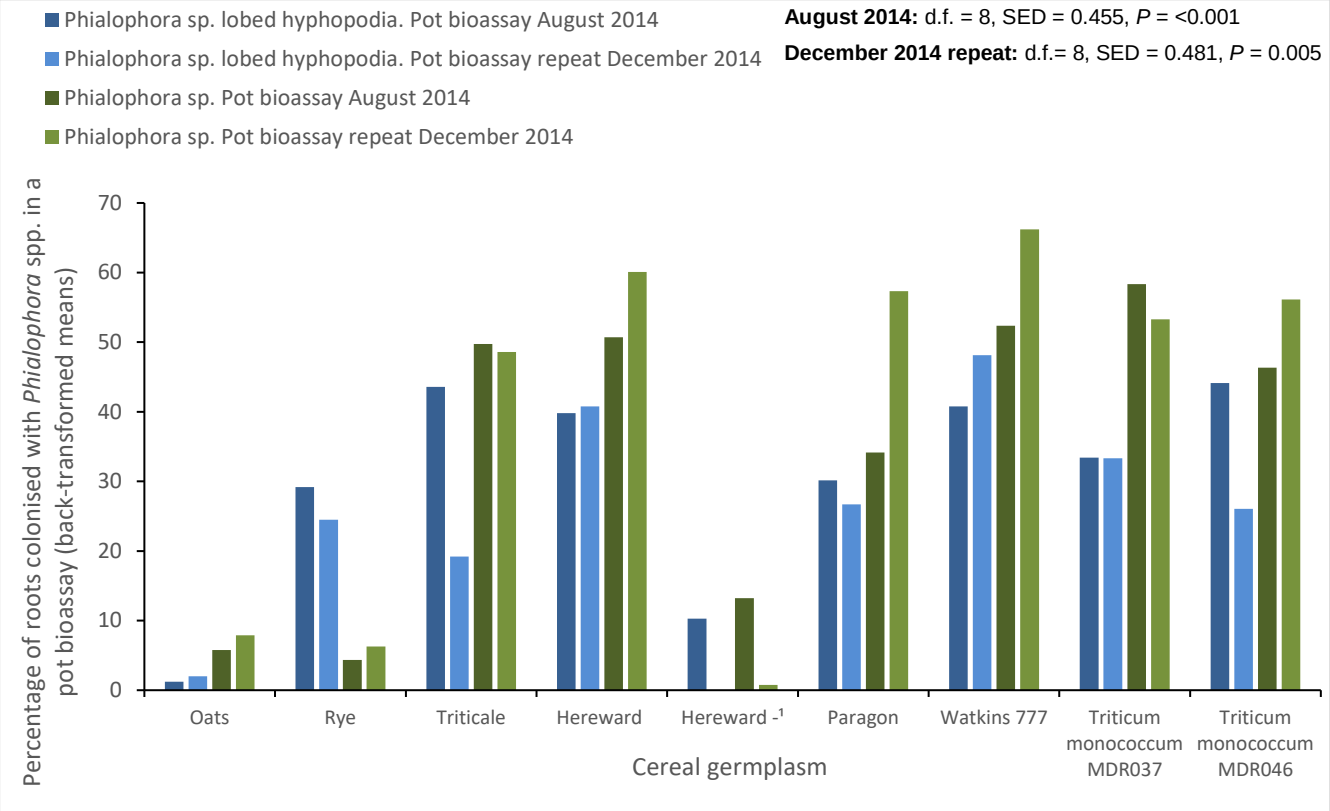

Figure 12. The ability of *Phialophora* species to colonise the seedling roots of cereal genotypes in a PDA inoculated pot bioassay for two experimental replicates.

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

4.5. *Phialophora* 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 4). 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 4). 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 4). 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.

The ITS5-ITS4 rDNA regions of the three *Phialophora* draft genomes were extracted and aligned. 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). 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).

Table 4. Genome assembly summary statistics of the three *Phialophora* draft genome sequences.

Genome summary statistics	***Phialophora* species**		
	***Phialophora* sp. lobed hyphopodia** **Isolate:** N.14.13 **Field:** New Zealand, RRes **Year:** 2013 **Crop:** spring barley, var. Tipple	***Phialophora* sp.** **Isolate:** S.09.13 **Field:** Summerdells I, RRes **Year:** 2013 **Crop:** wheat, var. Conqueror	***Phialophora graminicola*** **Isolate:** 74/1736-2 **Field:** Highfield, RRes **Year:** 1974[1] **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).

Table 5. 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

Table 6. 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

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 13). 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 13). 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

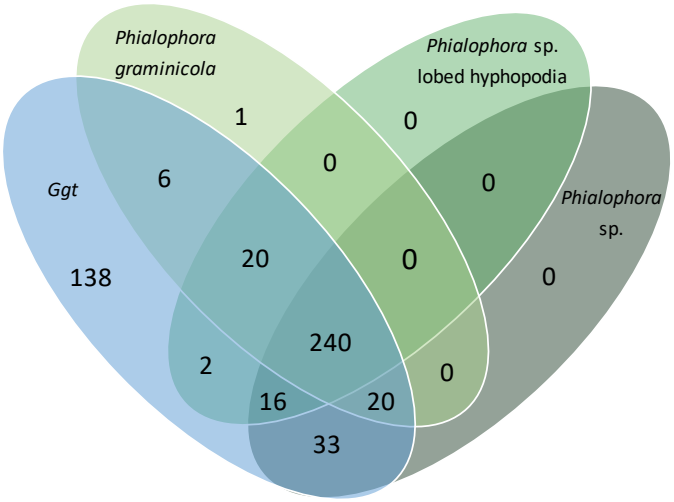

Figure 13. Unique number of complete single-copy BUSCOs of the 1,438 core fungal genes, excluding the complete duplicated BUSCOs, that were found in each of the individual *Phialophora* draft genomes or the *Gaeumannomyces graminis* var. *tritici* (*Ggt*) R3-111a-1 (annotated genome downloaded from Ensembl), that were specific to either two species or three species and that were common to all four fungal species.

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 6. 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.

4.6. First wheat field trial screening the AHDB Recommended List (2013/2014) of winter wheat varieties for their ability to build-up natural populations of *Phialophora* fungi

The soil core bioassays were assessed and a significant difference in the ability of the elite wheat varieties to build-up inoculum of natural populations of *Phialophora* species was found (Figure 14). *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) (Figure 14). Post-hoc grouping 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 14). 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 14). 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 14). 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 14). *Phialophora* spp. were isolated from colonised root tissue of the soil core bioassay seedling wheat plants from across the field site and the 19 isolates were identified as *Phialophora* sp. lobed hyphopodia.

The percentage data were then correlated against the nabim groups of the RL varieties to determine whether the percentage of roots colonised with *Phialophora* was affected by different grain qualities and uses of the four nabim groupings. 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 were also correlated against the year that the variety was first listed on the AHDB RL, the year data were ranked from the oldest to the newest varieties. 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). Yet a weak to moderate 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).

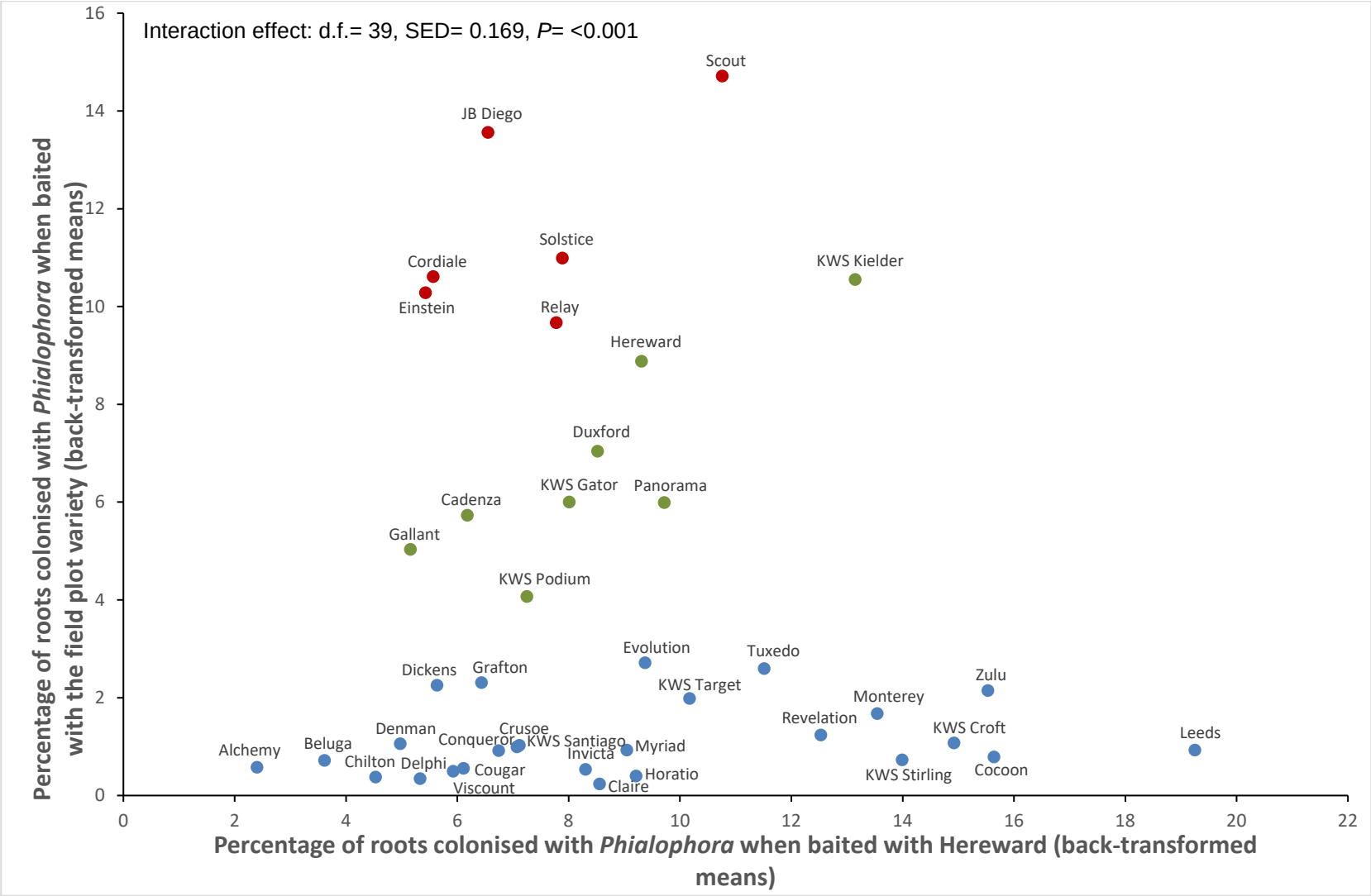

Figure 14. Correlation between the percentage of roots colonised with *Phialophora* when baited with either Hereward or the field plot variety in a soil core bioassay after the harvest of the elite wheat varieties of the first wheat 2014-2015 field trial. The elite wheat varieties were visually divided 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 plot variety (illustrated in red).

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 (Figure 15). 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) (Figure 15). 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).

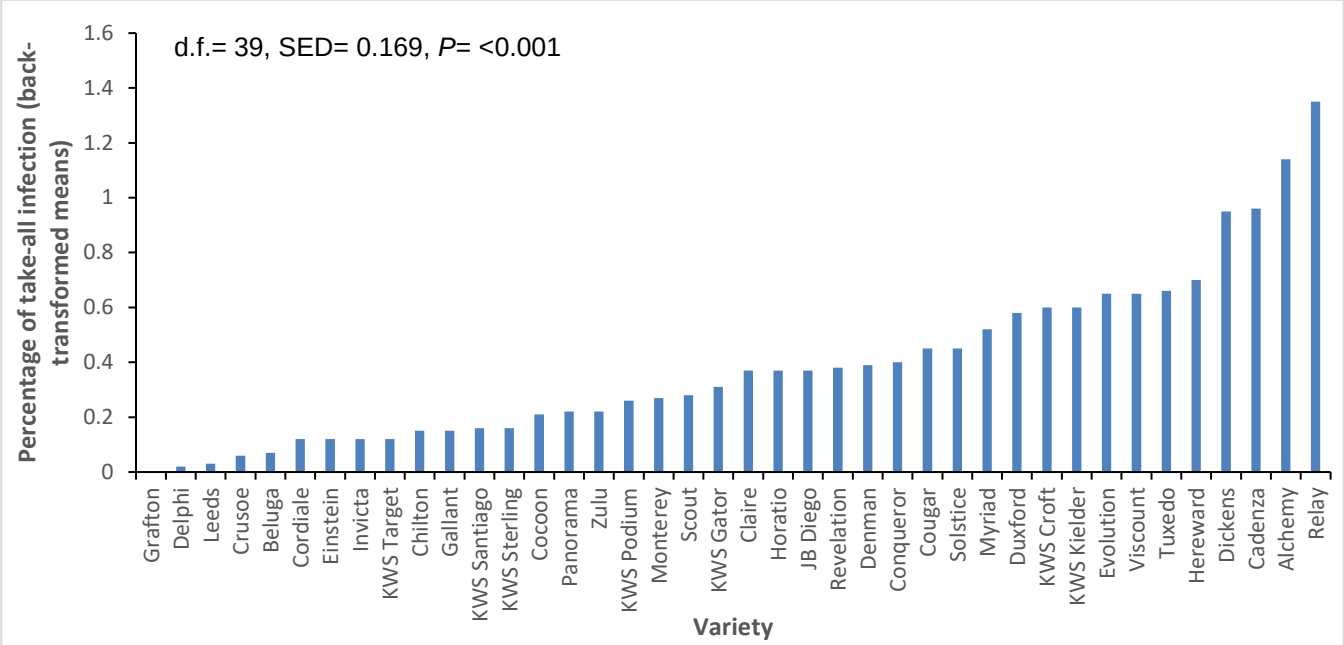

Figure 15. The percentage of roots infected with take-all in a soil core bioassay after the harvest of the elite wheat varieties of the first wheat field trial in 2014-2015.

There were significant effects of variety on days to anthesis (d.f.= 39, SED= 0.74, *P* = <0.001), days to leaf senescence (d.f.= 39, SED= 1.226, *P* = <0.001), plant height (d.f.= 39, SED= 1.308, *P* = <0.001) and plot yields (d.f.= 39, SED= 0.824, *P* = <0.001) (Figure 16). 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 (r_s = 0.427, *P* = 0.001, n = 40). 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, suggesting that the high level of *Phialophora* colonisation is not resulting in a detrimental effect on the overall grain yield. There was a moderately positive correlation between the percentage of roots colonised with *Phialophora* when baited with Hereward and plant height (Hereward x 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) but no significant correlation was found when baited with the field plot variety (r_s = -0.171, *P* = 0.073, n = 40).

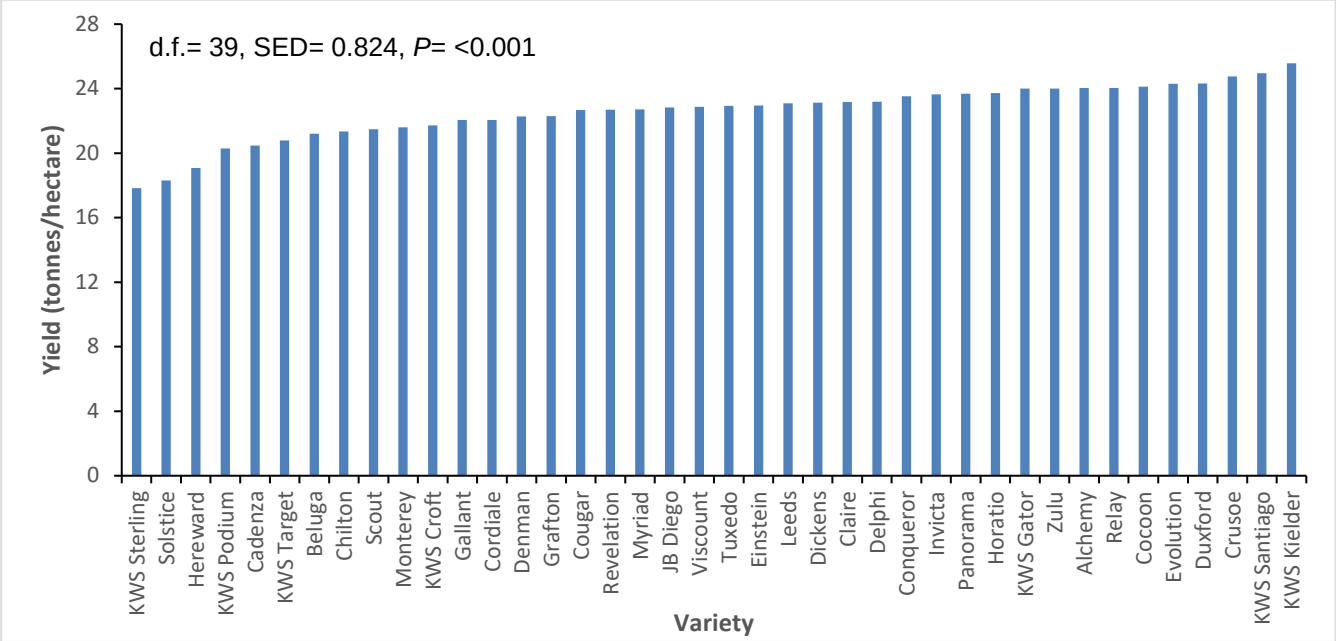

Figure 16. Yield of field plots screening the ability of *Phialophora* spp. to build-up under a first wheat crop in the 2014-2015 field trial.

5. Discussion

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 explored.

The most important finding within the take-all resistance 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. This provides the first known field screening of a *T. monococcum* mapping population to the susceptibility of take-all disease. The two third wheat field trials revealed that one line had a consistently low level of take-all infection across the two field seasons, a lower susceptibility than the resistant parental accession MDR046. 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 the two third wheat field trials screening the most take-all resistant hexaploid varieties. Of the five varieties with inserted soil moisture sensors, the three hexaploid varieties only exhibited resistance to take-all during the low take-all disease pressure field season of 2014-2015. 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. Another limitation was that only one sample point at the end of the season was assessed for take-all levels. Future work could look at taking plant samples throughout the spring and summer months, allowing a direct correlation with soil water for individual dates to help account for weather conditions on the build-up of take-all. 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. 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 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 seasons. Elite varieties such as JB Diego, Scout and KWS Kielder have the potential to maintain populations of *Phialophora*. In particular, the elite varieties Scout and KWS Kielder indicated the highest level of *Phialophora* spp. colonisation independent of baiting method. Potentially, some elite varieties could be used 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. 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. 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 several newer 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. 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). Take-all infection was found to be extremely low throughout the first wheat 2014-2015 field trial in this study, an average of 0.39% infected roots, and there did 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 these data are 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 high yield was seen across the first wheat field trial (17.83 - 25.57 tonnes/hectare) and there appeared to be no evidence of a detrimental effect of *Phialophora* colonisation on the yield of the plots. Wong et al. (1996) conducted field trials and found that the grain yield of wheat inoculated

with *Phialophora* sp. lobed hyphopodia was not significantly different to the yield of uninoculated wheat in three out of four field experiments.

A working pot bioassay protocol to screen the ability of *Phialophora* spp. to colonise the roots of additional cereal genotypes was devised from the AHDB summer bursary project in 2014 with undergraduate student Brittany Burton. 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). 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.

Arguably as important, was the development of a draft genome for three *Phialophora* species. A UK *Phialophora* isolate collection was gathered and from this an isolate representing each of the three *Phialophora* spp. was sequenced and the genomes were assembled and annotated. Genome comparisons revealed poor completeness values across the three *Phialophora* draft genomes compared to the *Ggt* genome, highlighting the difficulties of completeness with draft genomes. 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 the colonisation/infection process. The draft genomes will also allow a species-specific diagnostic assay to be developed in the future, which could allow *Phialophora* species to be detected in the farmers' fields.

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