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Student Report No. 31

Identification and characterisation of resistance to the take-all fungus in wheat

Vanessa Elizabeth McMillan

Department of Plant Biology and Crop Science, Rothamsted Research, Harpenden AL5 2JQ

Rothamsted Research supervisors: Mr Richard Gutteridge and Professor Kim Hammond-Kosack
Academic supervisor: Professor Nick Talbot, School of Biosciences, The University of Exeter

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CONTENTS

1. Abstract

Take-all disease, caused by the soil-borne fungus *Gaeumannomyces graminis* var. *tritici*, is the most devastating root disease of wheat around the world. Typical take-all symptoms show as black necrotic lesions on the roots and when severe can cause premature ripening and stunting of the wheat crop, resulting in poor grain quality and yield loss. Both cultural and chemical control methods are moderately successful at controlling take-all. Identifying plant material that would be useful for take-all control via a genetic approach has not been successful in the UK or elsewhere. The main aim of this project was to identify resistance to take-all within wheat (*Triticum* spp.).

This study explored a new phenomenon in hexaploid wheat (*Triticum aestivum*) which restricts take-all inoculum build-up (TAB) in the soil during a first wheat crop and also explored tissue-based resistance to take-all. Forty-nine elite wheat varieties were evaluated for their ability to build-up take-all inoculum in first wheat field trials using a soil core bioassay method. The effect of a low or high TAB first wheat variety on take-all disease and yield in a following second wheat crop was evaluated in crop rotation field trials. This work demonstrated that there are significant differences between current elite wheat varieties screened for the TAB trait and that there are probably multiple genetic sources of the trait. Take-all disease was lower and yields generally higher in a second wheat crop after a low TAB first wheat.

The susceptibility of the hexaploid wheat varieties to take-all was evaluated in third wheat field trials. No variety was highly resistant but the variety Hereford displayed some potential partial resistance to take-all. The ability of wheat varieties to build-up take-all inoculum in the soil during a first wheat crop was not related to their susceptibility to take-all root infection in the third wheat field trials. The implications of these new findings for the control of take-all via two distinct plant genetic approaches are discussed.

2. Introduction

2.1. Take-all disease of wheat

Take-all, caused by the soil-borne fungus *Gaeumannomyces graminis* var. *tritici* (*Ggt*), is the most serious root disease of wheat (*Triticum aestivum*) around the world. *Gaeumannomyces graminis* var. *tritici* is also able to infect other cereal species such as barley, rye and triticale. The typical take-all symptoms on wheat plants are root and stem base blackening. In the field above-ground symptoms show as stunted, yellow and prematurely ripened patches of wheat plants. Take-all restricts the ability of infected plants to take up water and nutrients from the soil and, as a result, there can be significant yield losses and poor grain quality (Hornby *et al.*, 1998).

Take-all disease is the classic example of a pathogen that builds up during short rotations, greatly reducing the attainable yield in successive wheat crops. In the first wheat crop in a rotation, take-all is usually negligible provided that the break crop before this is free from wheat volunteers and grass weeds such as the annual brome grasses (*Bromus* spp.) which are known to maintain take-all inoculum in the soil (Gutteridge *et al.*, 2006). Although take-all root infection is very low in a first wheat crop, large amounts of take-all inoculum can build up in the soil/rhizosphere region underneath this crop putting a following second wheat at risk from disease. Take-all is usually most severe in the 2nd to 4th wheat crops in the rotation and then typically declines if wheat continues to be grown. This latter phenomenon is known as take-all decline (TAD) (Slope & Cox, 1964) and is thought to be due to the action of antagonistic micro-flora in the soil.

In the UK, wheat is the principal cereal crop and each year approximately 35% of the total wheat crop area is sown as non-first wheat crops and so is at risk from take-all disease (Hornby *et al.*, 1998). Second wheats in the rotation usually yield around 1 to 1.5 tonnes/ha less than first wheat crops (HGCA Recommended List, www.hgca.com/varieties), and this is largely thought to be due to the effect of take-all disease.

Take-all disease also contributes towards the environmental issue of nitrate pollution. Severely infected wheat plants take up less nitrogen from the soil, leaving behind excess unused nitrogen fertiliser (mostly in the form of nitrates) (Macdonald & Gutteridge, 2012). This unused nitrogen is then available for leaching from farmland into surrounding waterways and ground water reserves. In England, approximately 60% of the land area (including all the main wheat growing regions) is designated as nitrate vulnerable zones (NVZs) (Environment agency, http://www.environment-agency.gov.uk/). In these areas, codes of good agricultural practice are imposed in order to minimise nitrate losses due to agriculture. The control of take-all is particularly important in these areas to minimise this added risk of excess nitrates in the soil.

2.2. Control of take-all

Take-all is one of the most difficult and important pathogens of wheat to control. Cultural and chemical controls can help to reduce take-all but currently there is no form of genetic control of take-all available to farmers.

Crop rotation is the most effective form of control. If a break crop is included in the rotation (such as oilseed rape, peas, beans or oats) then the risk of disease in the following first wheat crop is virtually absent (Yarham, 1981). This is due to the poor survival of take-all in the absence of a host plant. However, due to economic reasons, there is a large proportion of wheat and cereal-based rotations in the UK, so take-all remains a major constraint on productivity.

Other cultural control methods which can help to minimise yield losses due to take-all include:

- effective control of wheat volunteers and grass weeds in break crops
- delayed sowing of crops at risk from disease until mid-October (causes a decline in *Ggt* inoculum in the longer inter-crop period and a shorter time period for autumn infection) (Gutteridge & Hornby, 2003)
- good crop nutrition – phosphate, potassium, manganese and sulphur deficient soils are regarded as exacerbating take-all (Hornby *et al.*, 1998)

In terms of chemical control available to farmers there are two seed treatment fungicides fluquinconazole (commercially available as Jockey® since 2000) and silthiofam (commercially available as Latitude® since 2001) which have activity against take-all. However, fluquinconazole is not very effective against severe outbreaks of disease (Bateman *et al.*, 2004) and some take-all isolates are naturally insensitive to silthiofam (Carter *et al.*, 2003). Both seed treatments are also quite expensive. Two strobilurin fungicides, azoxystrobin and fluoxastrobin, applied during stem extension are also recommended for the control of take-all. However, results with azoxystrobin are sometimes inconsistent, varying widely in different field experiments (Bateman *et al.*, 2006).

Around the world, a variety of bacterial and fungal microorganisms have been isolated from the soil or wheat roots and examined in glasshouse and field trials for their potential biological control of take-all. Often results have not translated from glasshouse to field experiments. Bio-control in an agricultural field is a big challenge due to the heterogeneous nature of the soil environment and large number of potentially interacting microorganisms present in the soil. Until now, the biological control of take-all is often inconsistent in the field and so not considered economically viable (Weller *et al.*, 1988; Cook, 2003). In the UK there are no commercially available bio-control products against take-all.

Considering the only partial success of current control methods for take-all, the identification of resistant plant material as a means to control disease would be a big advantage because it could reduce the use of pesticides, provide more durable disease control and give farmers more freedom in rotational cycles. There is extensive literature on the search for resistance to take-all in wheat (Scott, 1981; Hornby *et al.*, 1998). The search for resistant wheat material, useful in breeding programmes, has so far been unsuccessful. Other related species (such as rye and oats) display differences in their susceptibility to take-all although none so far have been successfully utilised to improve the resistance of wheat. In individual field experiments often relatively large differences have been reported between wheat varieties in their susceptibility or tolerance to take-all. However, for any partial resistance to be useful it needs to be consistently expressed between sites and seasons in the field. Often the relative resistance of particular varieties has not been confirmed in further seasons or has not be considered big enough to be useful in wheat breeding programmes for the control of take-all (Scott, 1981). There is no specific information on the susceptibility of current winter wheat varieties to take-all, but modern wheat as a whole is considered fully susceptible to the disease.

There is limited evidence that some wheat varieties may be more tolerant of take-all disease, performing relatively well in terms of yield as second wheats compared with other varieties. This is apparent in the HGCA Recommended List (RL) first and second wheat yield trials (www.hgca.com/varieties), where some varieties have quite large second wheat yields compared to the control variety. However, RL trials are carried out on different sites, making the interpretation of this data more difficult, as yield typically has a large variety x site interaction.

In contrast to the generally limited differences in susceptibility of wheat varieties to take-all root infection there was one UK report in the 1980s that suggested that wheat varieties built up differing amounts of take-all inoculum in the soil during the first wheat crop in the rotation (Widdowson *et al.*, 1985). This was investigated more recently within the Defra funded Wheat Genetic Improvement Network (WGIN; www.wgin.org.uk) programme and in the initial year of my PhD. In this study we detected consistent differences between a wide range of wheat varieties in their ability to build-up take-all inoculum in the soil beneath the first wheat crop (McMillan *et al.*, 2011). We called this trait LowTAB (Low **T**ake-**a**ll inoculum **b**uild-up). This could potentially reduce the risk of severe take-all in a following second wheat crop.

Before the start of the PhD project, a small number of winter wheat varieties were also assessed for their susceptibility to take-all root infection in pot and field experiments. Unexpectedly, statistically significant differences were found in the severity of disease between different varieties. This was at the same time as work from the WGIN programme was showing differences between older semi-modern varieties in their ability to build-up take-all inoculum in the soil in a first wheat crop (see above). In light of this information the take-all inoculum building ability and susceptibility of current commercial UK National or Recommended List varieties (in 2008/2009) was examined in a series of first and third wheat field trials for a thorough study of the interactions between modern wheat varieties and take-all.

2.3. Project aims

The overall aim of the PhD project was to identify sources of resistance to the take-all fungus which could be exploited in wheat breeding programmes to improve the control of take-all. The project can be split into two areas:

1. Investigating the take-all inoculum building ability of modern commercial winter wheat varieties (TAB trait). This was carried out under field conditions by using a soil core bioassay method to gauge the amount of take-all inoculum in the soil after harvest of first wheat variety trials. In addition variety rotational trials were used to investigate the value of this trait to reduce take-all and improve yields in second wheat crops.
2. Exploring the susceptibility of modern commercial winter wheat varieties to take-all root infection. This was carried out by taking plant samples for disease assessment and yields from third wheat (at risk from severe take-all disease) variety trials.

3. Materials and methods

Field trials were all set up on the Rothamsted farm (Hertfordshire, UK) on flinty clay loam soil of the Batcombe soil series. Trials were sown at 350 seeds/m² and throughout the growing season growth regulator, pesticides, and fertiliser were all applied according to standard Rothamsted farm practice, except that no take-all seed treatments or fungicides were used. Yields were taken from all trials by the Rothamsted farm.

3.1. First wheat trials: Take-all inoculum build-up trait

Three first wheat field trials, in the harvest years of 2009, 2010 and 2011, were used to study the take-all inoculum building ability of 45 previous, current or candidate HGCA RL winter wheat varieties. These were sown in the autumn after a one or two year break away from wheat, i.e. winter oats or field beans to reduce take-all inoculum in the soil to negligible amounts before the start of the experiment. The same varieties were sown in all three years apart from three changes in the second year and one additional change in the last year (this was because seed of the original varieties was no longer available). Trials were arranged as alpha designs with incomplete sub-blocking within the four main blocks/replicates of the 45 varieties in each year. All trial designs were generated by the Rothamsted Statistician Rodger White using CycDesigN (VSN International Limited, Hemel Hempstead, UK).

After harvest a soil core bioassay method (Slope *et al.*, 1979) (Figure 1) was used to evaluate the TAB trait. Soil cores (eight per plot) were taken after harvest of the three first wheat field trials. Cores were upturned into plastic cups, transported back to the field laboratory and ten wheat seeds of our standard wheat variety (Hereward) placed on the surface of the soil. The surface of core and seeds were covered with horticultural grit and the cups placed in a controlled environment room for 5 weeks (day/night temperatures 15/10°C). After five weeks the wheat seedlings were washed free of soil and assessed for take-all lesions in a white dish under water. The total number of roots and the number of diseased roots were counted for each soil core so that the percentage of roots infected could be calculated. This is a gauge of the amount of infective take-all inoculum that has built up in the soil under the different first wheat varieties and so the potential risk of take-all disease if a following second wheat crop was sown in the field.

Figure 1. Soil core bioassay method (Slope *et al.*, 1979).

Plant samples were also taken from each field trial for take-all disease assessment. This was to check that there were only very minor levels of take-all root infection as should be expected in a first wheat crop. These samples were taken in the summer between Growth Stage (GS) 73-83 (grain filling); samples were dug from five 20 cm lengths of row per plot (approx. 40-50 plants per plot). Samples were washed free from soil, air-dried and then stored at room temperature before take-all disease assessment over the winter months. Take-all was assessed by first soaking the dried plant root systems in water for at least 15 minutes before grading (by eye in a white dish under water) each whole plant root system into categories from slight to severe based on the proportion of roots infected. From this a take-all index (TAI) from 0 (no take-all) to 100 (all plants severely infected) was calculated (Bateman *et al.*, 2004).

For the 2009 and 2010 field trials monthly epidemiology studies on the build-up of take-all inoculum under different varieties were carried out as part of HGCA-funded summer bursary projects with students James Bruce (2009) and Nicola Phillips (2010). These studies were conducted for six selected wheat varieties within the trials (Table 1). These varieties were chosen to give a range of TAB phenotypes based on their performance within the earlier WGIN trials (McMillan et al., 2011). Five soil cores per plot were taken at monthly intervals from March or April until harvest when the final soil cores were taken from all plots. The aim was to identify the critical time period(s) during which differences between varieties occurred in the field.

Table 1. Varieties selected for epidemiology studies in the 2009 and 2010 first wheat elite variety field trials.

Variety	TAB[1]
Avalon	high
Cadenza	low
Cordiale	low
Hereward	high
Riband	medium
Xi19	low

[1] Take-all inoculum build-up (TAB), varieties classified based on performance in WGIN field trials (McMillan *et al.*, 2011).

3.2. Rotation trials

Two 2-year wheat variety rotation trials were set up as first wheats in the rotation in autumn 2008 and autumn 2009 to explore the effect of differences in take-all inoculum build-up in a first wheat on take-all disease and yields in a following second wheat crop. In Year 1 the trials consisted of 4 replicates of the winter wheat varieties Cadenza (low TAB variety) and Hereward (high TAB variety), sown as large 12m x 82m plots. In the second year each of the large plots was divided into eight 3m x 10m plots and sown with 8 different elite wheat varieties (Cordiale, Duxford, Einstein, Gallant, Hereward, Robigus, Solstice and Xi19).

After harvest in the first year the soil core bioassay method (described in section 2.1) was used to gauge the infectivity of the soil. Five soil cores per plot were taken from the location of each of the following year’s plot positions. In the second year plant samples were taken in the spring (five 15 cm lengths of row per plot) and summer (ten 20 cm lengths of row per plot) for take-all disease assessments (described in section 2.1.)

3.3. Third wheat trials: Susceptibility to take-all root infection

Third wheat trials (in the harvest years 2009, 2010 and 2011), after two previous winter wheat crops, were using to investigate the susceptibility of modern elite winter wheat varieties to take-all root infection at an expected high natural take-all disease pressure. In 2009 and 2010 the trials were arranged as alpha designs (the same as the first wheat trials) with four replicates of 45 elite winter what varieties. In 2011 the number of varieties was reduced to 12 based on the results from the first two years.

Plant samples were taken in the spring (five 15cm lengths of row per plot) and summer (ten 20cm lengths of row per plot) for take-all disease assessment as described in section 2.1.

3.4. Statistics

Spearman’s rank was used on raw data to assess associations between different disease variables. Data was then statistically analysed by the Rothamsted Statistician Rodger White. Percentage disease data was transformed to logits before further analysis. The three first wheat field trials and the 2009 and 2010 third wheat field trials were analysed using the REML procedure in Genstat to include the sub-blocking within these trials. The 2011 third wheat field trial did not include sub-blocking so results were analysed by analysis of variance. A combined year analysis of the three third wheat field trial results was carried out using REML. In the rotation trials analysis of variance was used to detect treatment effects and interactions between year 1 ‘source’ variety and year 2 ‘over-sow’ varieties. Significant effects were supposed when $p \leq 0.05$.

4. Results

4.1. First wheat trials: Take-all inoculum build-up trait

In 2009 significant differences were detected between a wide range of elite wheat varieties in their ability to build-up inoculum of the take-all fungus when sown as a first wheat crop ($P < 0.001$, Figure 2). Twelve of the forty-five varieties included in the 2009 trial had been previously evaluated for the TAB trait within the WGIN diversity field trials (McMillan *et al.*, 2011)(purple bars in Figure 2). Within these trials Cadenza and Xi19 were consistently some of the lowest inoculum builders. In contrast the varieties Avalon and Hereward were among the highest building varieties over multiple years of field trials. In the 2009 PhD field trial this also proved to be the case. However, not all of the previously tested varieties performed as would be excpected based on the results in the WGIN trials. For example within the WGIN trials the variety Malacca was generally a high TAB variety but in the 2009 PhD trial this was the lowest building variety out of the whole trial. These findings emphasise the need for multiple years of field testing to accurately identify those varieties with more stable phenotypes. Despite this the 2009 field trial does suggest that there could be potentially important differences between modern elite wheat varieties in the build-up of take-all inoculum in the soil.

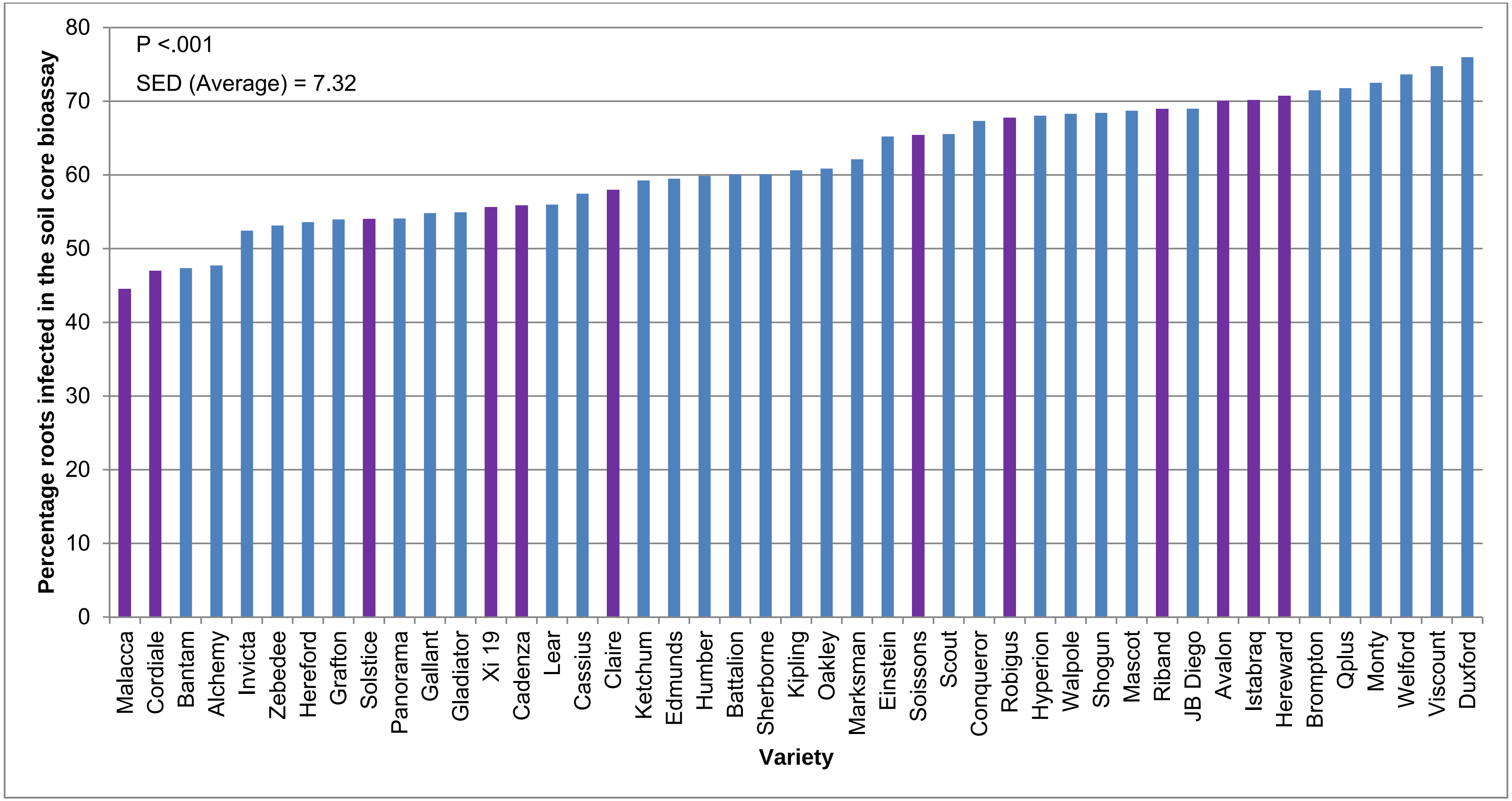


Figure 2. Take-all inoculum build-up after harvest of the 2009 elite winter wheat variety trial sown as a first wheat crop. Varieties in purple have been previously evaluated for the TAB trait within the WGIN diversity trials (McMillan *et al.*, 2011).

Plant samples were taken from the first wheat trial to confirm that there was only negligible take-all infecting the roots of the first wheat crop. This was the case with a mean take-all index across the whole trial of 1.02 (scale: 0-100) and no significant effect of variety (P = 0.427). There was also no correlation between the percentage of roots infected in the soil core bioassay and the take-all index of plant samples (Spearman's rank correlation [Rs] = 0.07, P = 0.35, n = 180). There was a slight significant negative correlation between yield in the first wheat trial and take-all inoculum build-up on a per plot basis (Rs = -0.28, P <.001, n = 180, Figure 3).

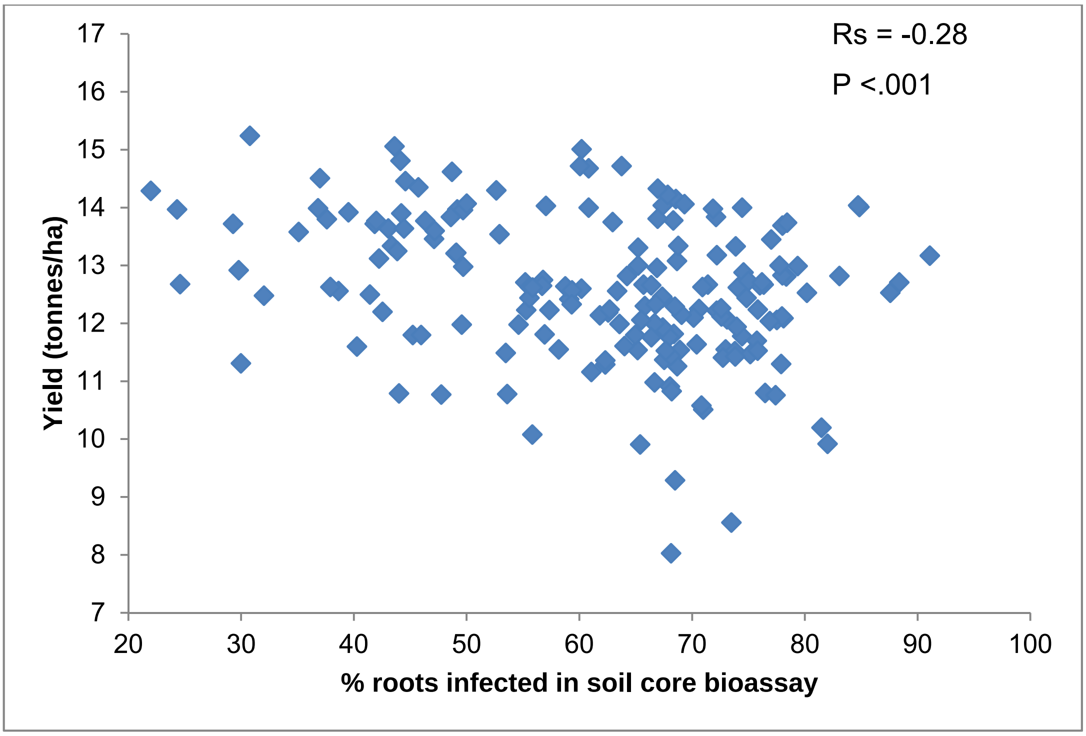


Figure 3. Per plot correlation between the percentage roots infected on bait plants in the soil core bioassay and yields in the 2009 first wheat field trial (09/R/WW/916).

The first wheat variety trial was repeated in 2010 and 2011 with the aim of evaluating how consistently these varieties perform over multiple years and different trial sites. Unfortunately varietal performance could not be confirmed in these trials because of unsuitable conditions for testing varieties. In 2010 take-all inoculum failed to develop across the whole trial site due to the extremely dry weather in the spring and summer. During some of the key months of inoculum build-up in April, June and July 2010 there was half the total rainfall than in 2009. This probably restricted the build-up of inoculum with the mean percentage roots infected in the soil core bioassay after harvest of only 0.6%.

In the 2011 trial, the presence of the naturally occurring, weakly parasitic and competing fungus *Phialophora graminicola* (Hornby et al., 1998) across parts of the site restricted the build-up of take-all inoculum (see Discussion, page 31). This resulted in a very patchy distribution of inoculum build-up making it unsuitable for detecting treatment effects.

4.2. Epidemiology studies

The monthly epidemiology studies in 2009 and 2010 show a baseline percentage of roots infected in the soil core bioassay of around 10% in April of both years (Figures 4 and 5). In 2010 sampling was started a month earlier in March. The soil was already infective at this time although now there was less than 5% roots infected in the soil core bioassay.

In the 2009 field season there were significant increases in the infectivity of the soil from May onwards ($P < 0.001$). Interestingly there was a trend for a ‘stall’ in inoculum build-up from June to July for the two high TAB varieties Avalon and Hereward, before a rapid increase between July and the after harvest sampling date in August. However, all six varieties generally show a similar time course of inoculum build-up, with no significant main effect of variety ($P = 0.342$) or interaction effect between variety and month ($P = 0.323$). A significant main effect of variety was only detected when all 45 varieties were sampled after harvest ($P < 0.001$, Figure 2).

In contrast to 2009 the infectivity of the soil in the 2010 trial declined from April to July (Figure 5). This is most likely due to the dry weather in the spring of 2010 which was unfavourable for take-all development. Under these conditions there was no effect of variety on TAB in the epidemiology study ($P = 0.508$) or in the main after harvest study of all 45 varieties ($P = 0.614$, see section 3.1. for main after harvest study).

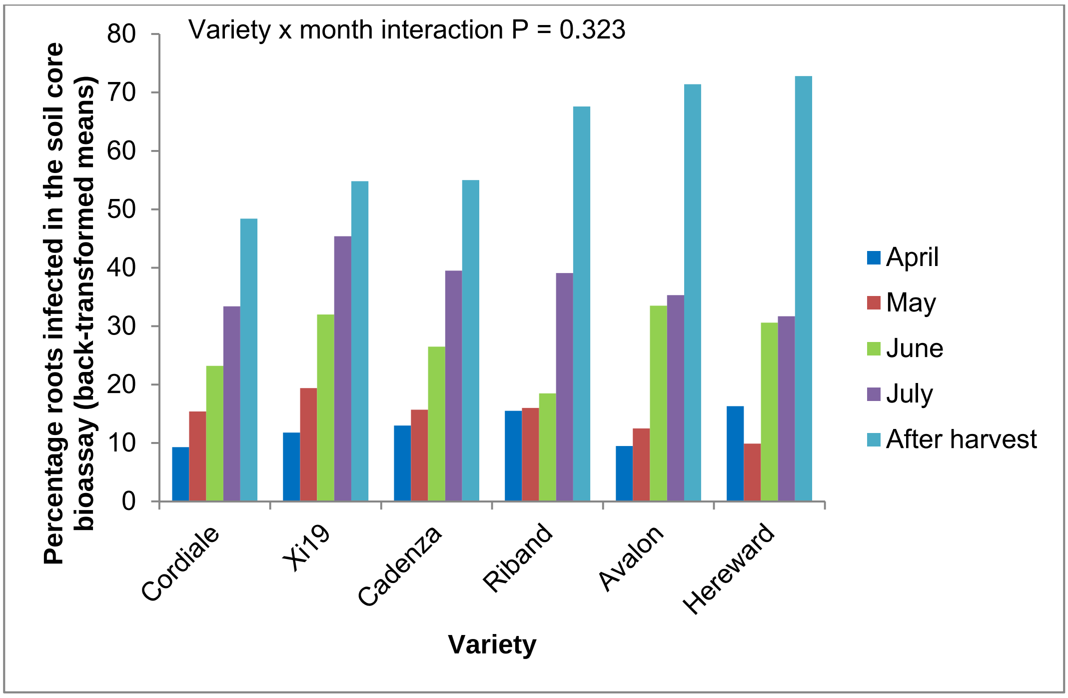


Figure 4. Epidemiology study on take-all inoculum build-up from April through to harvest under six winter wheat varieties in the 2009 elite winter wheat field trial.

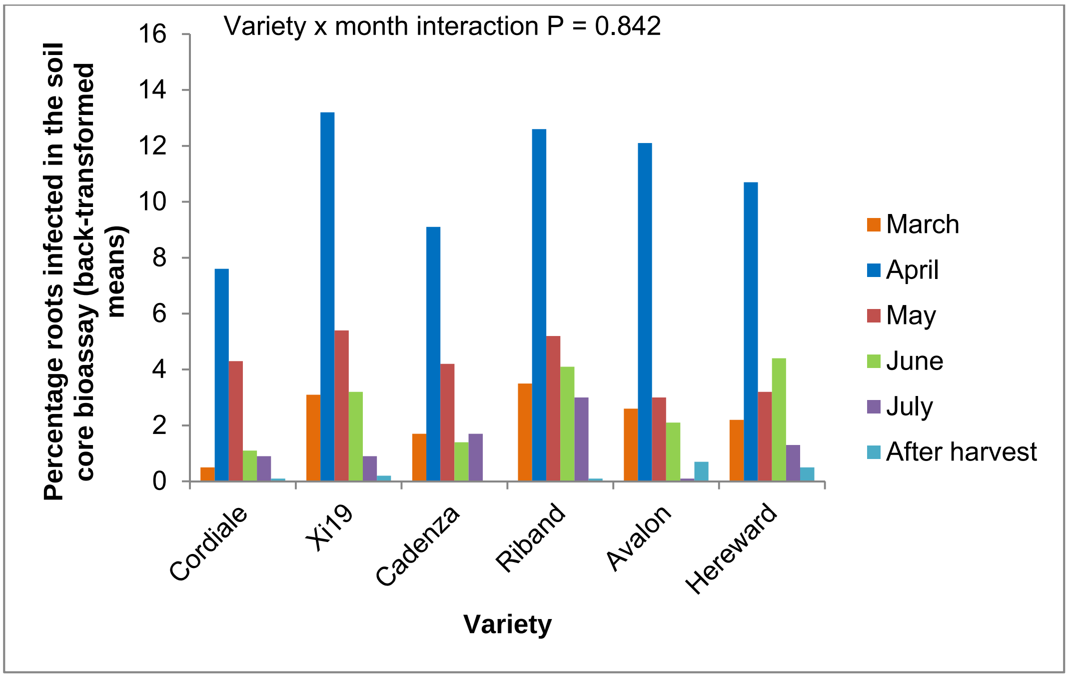


Figure 5. Epidemiology study on take-all inoculum build-up from March through to harvest under six winter wheat varieties in the 2010 elite winter wheat field trial.

4.3. Rotation trials

After harvest in year 1 (harvest year 2009) of the first rotation trial there was a significant difference in the take-all infectivity of the soil after the varieties Cadenza (low TAB in WGIN trials) and Hereward (high TAB in WGIN trials) (P = 0.027) (Figure 6). Low levels of the fungus *Phialophora graminicola* were also identified on the roots of the soil core bioassay plants throughout the whole trial. This probably restricted the build-up of take-all inoculum in comparison to the 2009 elite winter wheat field trial in the same year (see section 3.1. above for details of elite wheat trial, mean build-up of 61.7% roots infected in the soil core bioassay compared to 15.6% in rotation trial).

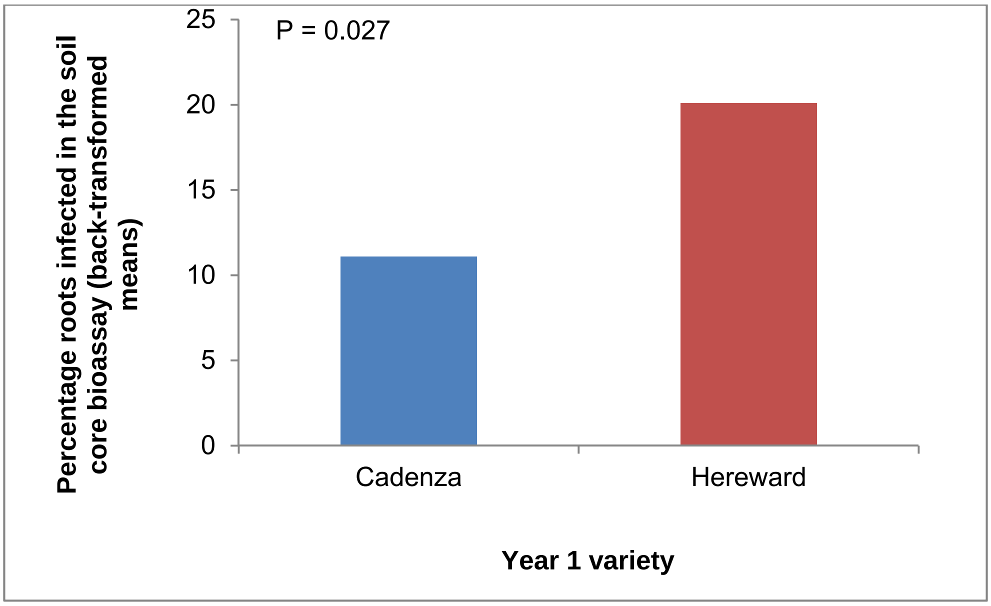


Figure 6. Rotation trial 1, year 1: Take-all infectivity of the soil after harvest of the wheat varieties Cadenza and Hereward sown as a first wheat.

In year 2 there was significantly more take-all disease for the eight second wheat varieties grown after Hereward compared with Cadenza in year 1 (Mean second wheat take-all Index (0-100) after Cadenza in year 1 = 8.3, after Hereward in year 1 = 18.5, P = 0.04). There was also a significant effect of year 2 over-sow variety (P = 0.002) (Figure 7). The variety Robigus was the most severely infected while Solstice had the lowest amount of take-all, demonstrating potential differences in the susceptibility of winter wheat varieties to take-all. Importantly there was no significant interaction effect indicating that the pattern of response of the eight second wheat varieties after Cadenza and Hereward was similar. The difference in the amount of take-all disease after the low TAB variety Cadenza or high TAB variety Hereward in year 1 was also reflected by the mean second wheat plot yields which were on average (for all eight varieties) 0.44 tonnes/ha lower after Hereward than Cadenza, although this was not significant (P = 0.19)(Figure 8). There were also differences, as expected, between the second wheat varieties, which have inherent yield differences even in the absence of take-all. Surprisingly the Robigus plots following Hereward were reasonably high yielding (Figure 8); despite the relatively severe take-all disease found in these plots (Figure 7).

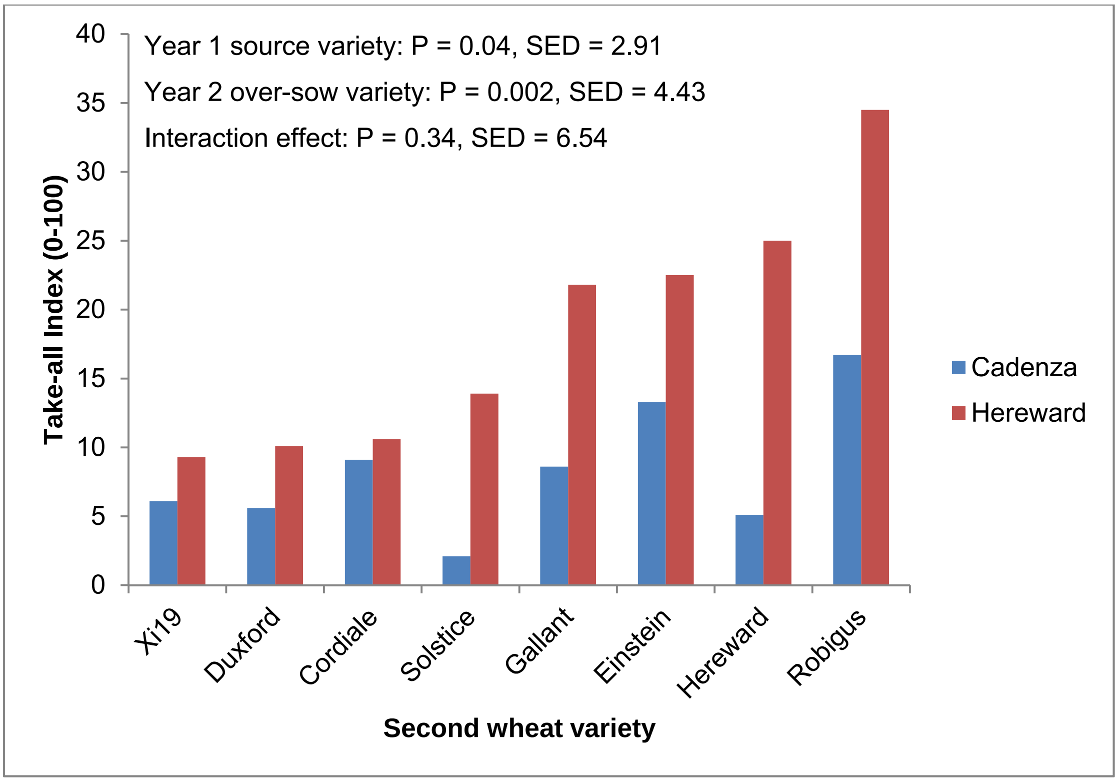


Figure 7. Rotation trial 1, year 2: Take-all severity in the summer for eight winter wheat varieties sown as a second wheat crop after the first wheat varieties Cadenza and Hereward.

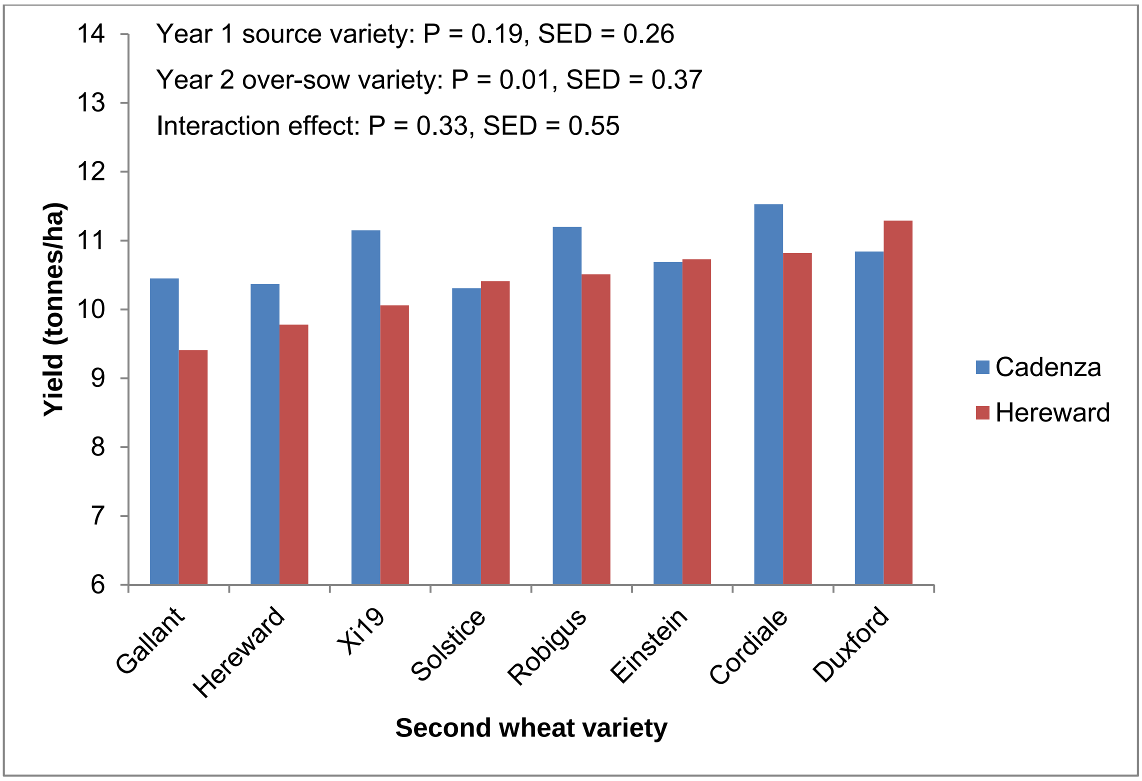


Figure 8. Rotation trial 1, year 2: Second wheat yield of eight winter wheat varieties sown after Cadenza or Hereward in year 1.

In the first year of the second rotation trial (harvest year 2010) there was only a very low level of take-all inoculum build-up across the trial site (mean 2.2% roots infected in the soil core bioassay). This was probably due to the lack of rainfall in the spring and early summer of 2010. Under these conditions there was no significant effect of the first wheat variety (Cadenza or Hereward) on take-all inoculum build-up (P = 0.45) (Figure 9).

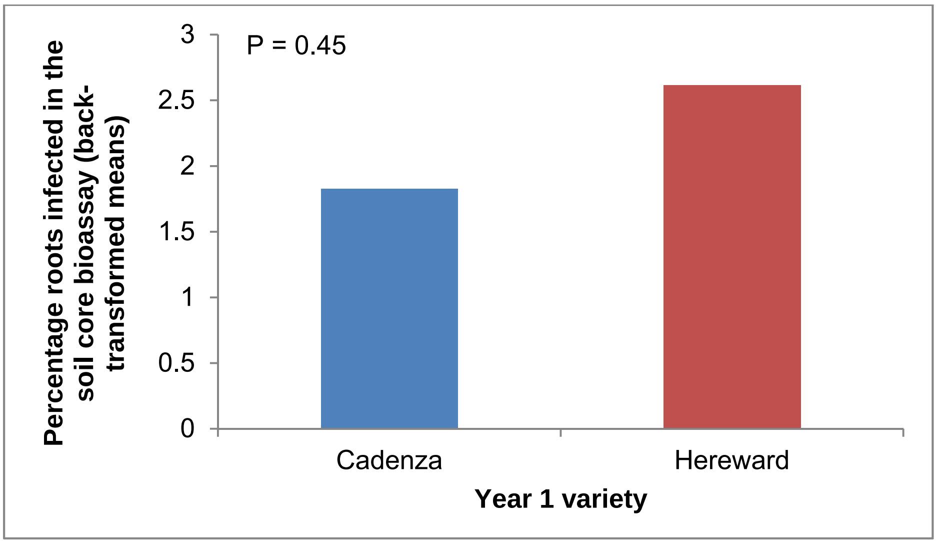


Figure 9. Rotation trial 2, year 1: Take-all infectivity of the soil after harvest of the wheat varieties Cadenza and Hereward sown as a first wheat.

Despite the low level of inoculum build-up there was still a trend for higher amounts of take-all disease severity in the spring and summer of year 2 after the Hereward plots than the Cadenza plots (Spring, mean number of take-all infected roots per plant after Cadenza = 0.052, after Hereward = 0.116, P = 0.039, Figure 10) (Summer, take-all index after Cadenza = 13.49, after Hereward = 21.07, P = 0.048, Figure 11). No significant interactions were detected between first and second wheat variety treatments. However, second wheat variety choice did have a significant effect on take-all disease severity at the summer sampling point. In agreement with the first rotation trial the variety Robigus was the most severely infected (Figure 11).

In the second year yields were 0.2 tonnes/ha lower after the high TAB variety Hereward than the low TAB variety Cadenza sown in year 1 (P = 0.043), providing evidence that sowing a low TAB variety in the first year of a rotation can help to reduce take-all and improve yields in a following second wheat crop. Again there was no significant interaction between first and second wheat variety treatment, although Duxford (which had the lowest take-all index in year 2) had a higher yield after the Hereward than Cadenza plots in year 1 (Figure 12).

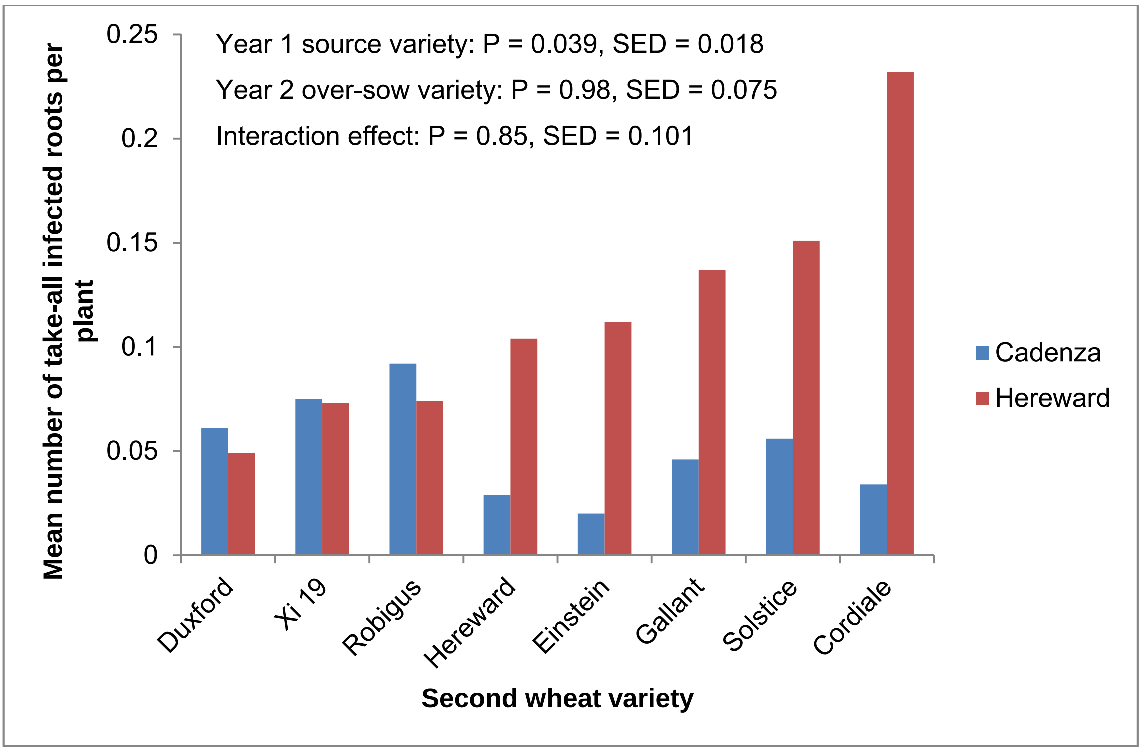


Figure 10. Rotation trial 2, year 2: Take-all severity in the spring for eight winter wheat varieties sown as a second wheat crop after the first wheat varieties Cadenza and Hereward.

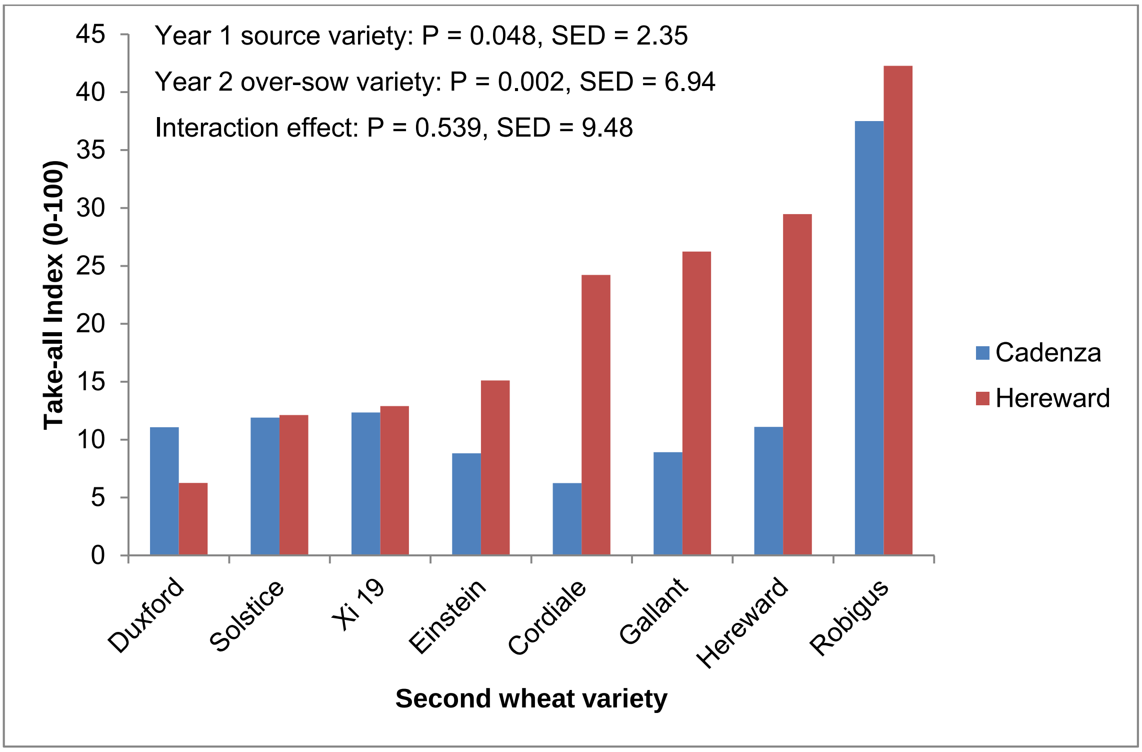


Figure 11. Rotation trial 2, year 2: Take-all severity in the summer for eight winter wheat varieties sown as a second wheat crop after the first wheat varieties Cadenza and Hereward.

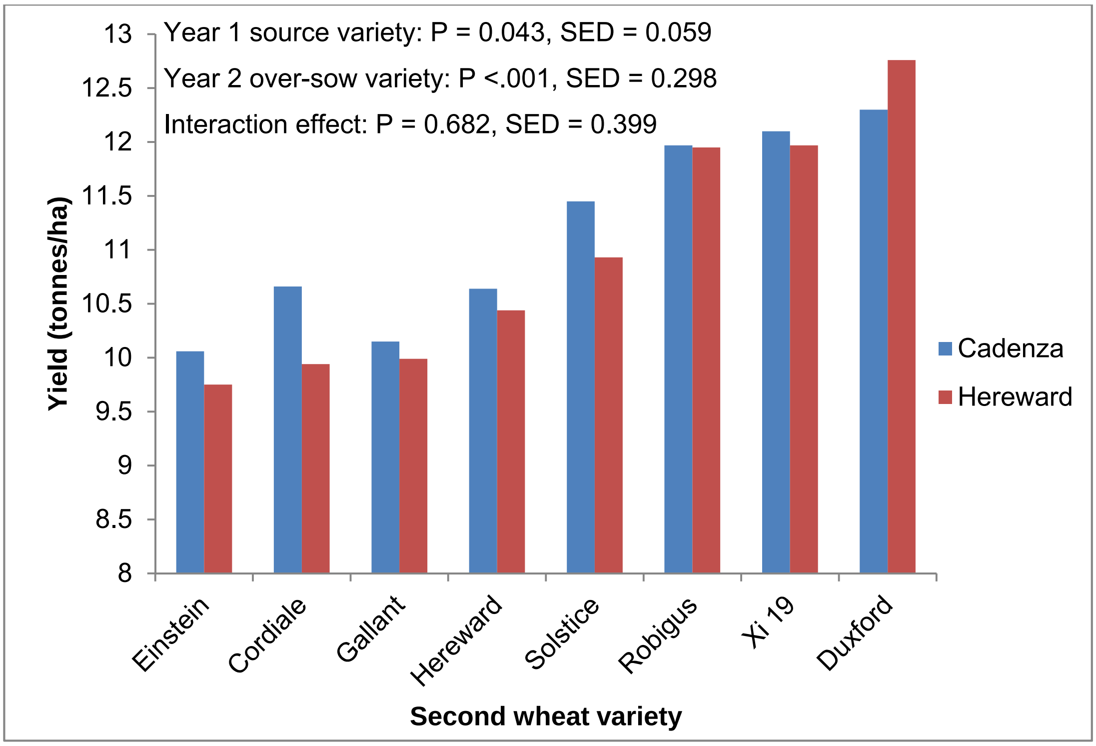


Figure 12. Rotation trial 2, year 2: Second wheat yield of eight winter wheat varieties sown after Cadenza or Hereward in year 1.

4.4. Third wheat trials: Susceptibility to take-all root infection

In the 2009 trial the incidence and severity of take-all in the spring was already high across the whole trial, with variety means ranging from 62.2% to 95.8% plants infected and 1.24 to 2.45 take-all infected roots per plant. There was no significant effect of variety on either disease variable (Logit % plants infected: P = 0.246; take-all infected roots per plant: P = 0.097).

In contrast significant varietal differences were detected at the summer sampling point for both the percentage of plants infected (P = 0.002) and the take-all index (P <0.001). Over 95% of plants were infected with take-all across the whole trial and there was a mean take-all index (0-100) of 74.07, with variety means ranging from 56.48 (cv. Hereford) to 83.86 (cv. Monty) (Figure 13).

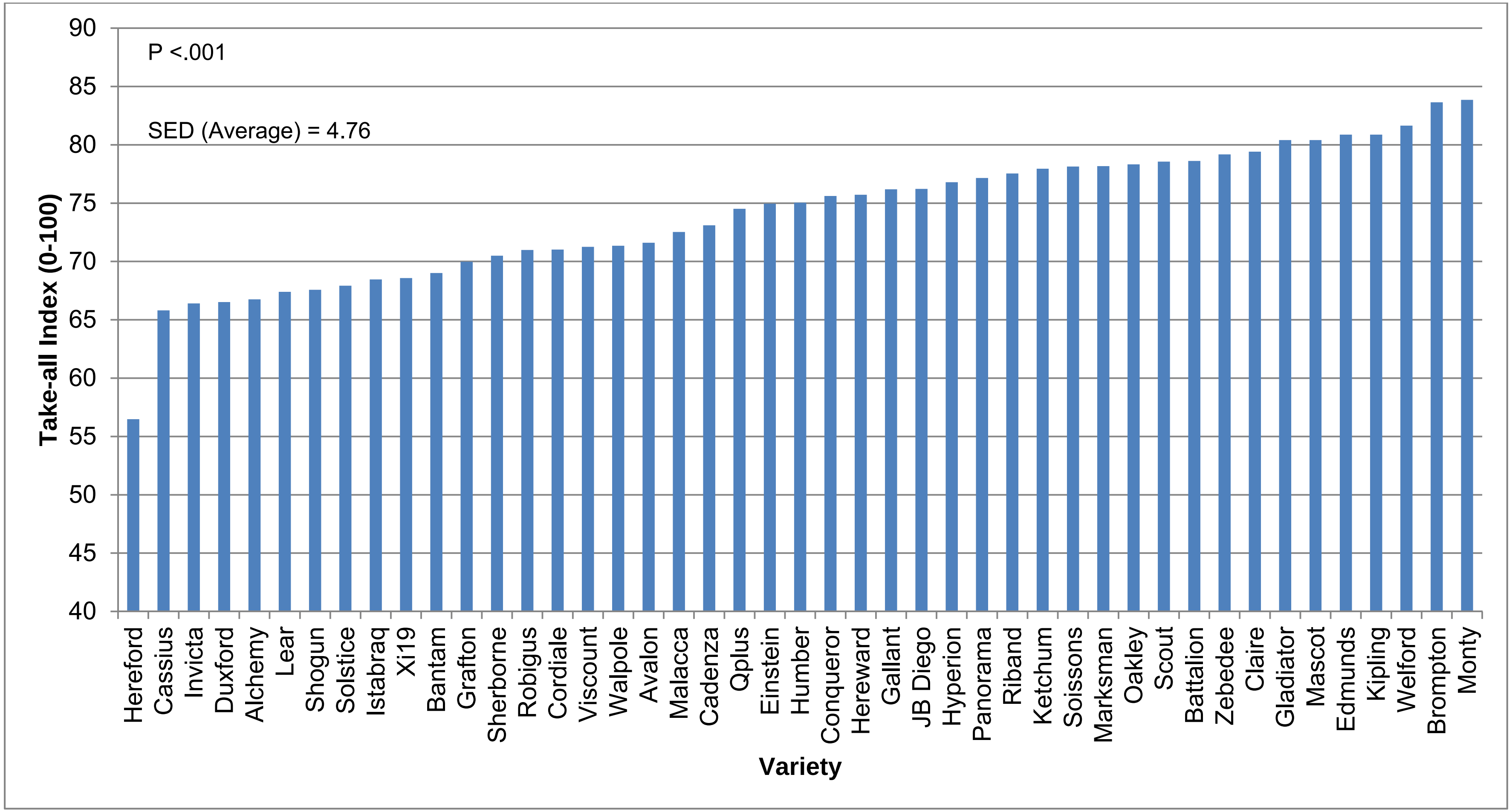
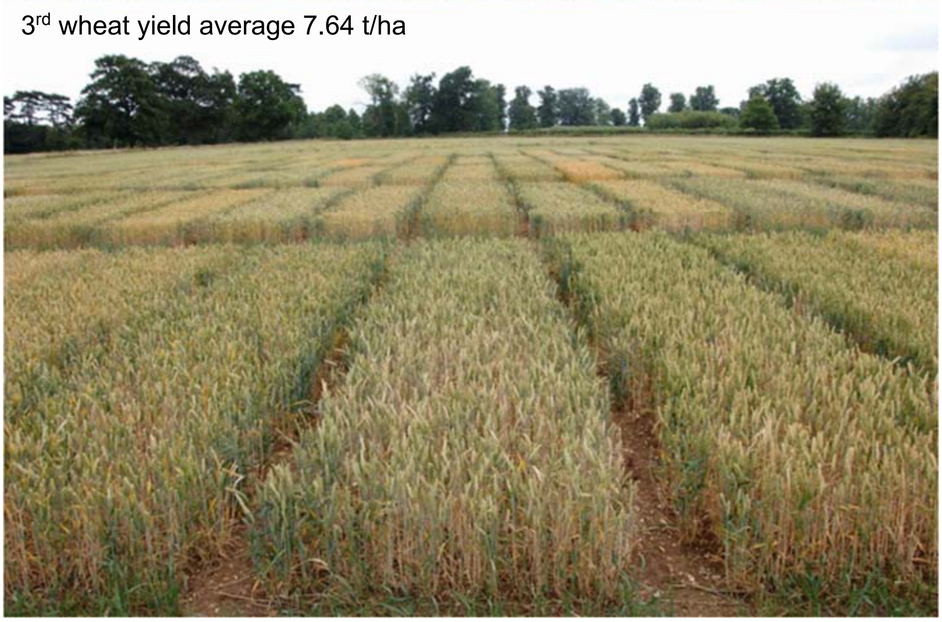


Figure 13. Take-all disease severity in the summer of the 2009 elite winter wheat variety trial sown as a third wheat crop.

As expected, there was a highly significant effect of variety on yield ($P < 0.001$). Yields were also recorded for the first wheat field trial (with negligible take-all root infection) which included the same 45 varieties x 4 reps (see section 3.1.). The percentage yield loss between the first and third wheat trials could then be calculated for each variety to identify the varieties which perform best under a high take-all disease pressure. Overall yields were 39.15% lower in the third wheat trial compared the first wheat trial. This was also reflected in the poor above ground appearance of varieties in the third wheat trial (Figure 14). Overall there was a significant relationship between variety yields in the first and third wheat field trials ($R_s = 0.54$, $P < 0.001$, $n = 45$). The winter wheat variety Hereford, which had the lowest take-all index in the third wheat trial, had the highest mean 3rd wheat yield. However, Hereford was also one of the highest yielding varieties in the first wheat trial so its percentage yield loss is similar to those of other varieties (Figure 15). Percentage yield loss was not correlated with the take-all index ($R_s = 0.22$, $P = 0.14$, $n = 45$).

The susceptibility of the 45 elite wheat varieties to take-all root infection in this third wheat trial could also be compared to their ability to build-up take-all inoculum in the soil in the first wheat field trial in the same year. There was a significant effect of variety in both trials for the two different traits but a correlation analysis revealed no strong significant correlation between the traits ($R_s = 0.28$, $P = 0.06$, $n = 45$) (Figure 16).

1st wheat yield average 12.69 t/ha



3rd wheat yield average 7.64 t/ha

Figure 14. Photographs of the 2009 first wheat (top picture) and third wheat (bottom picture) variety field trials on the Rothamsted farm. Both trials sown on 9th October 2008. Photographs taken on 8th July 2009. Above ground symptoms of severe take-all disease (prematurely ripened and stunted plants) visible in the third wheat trial.

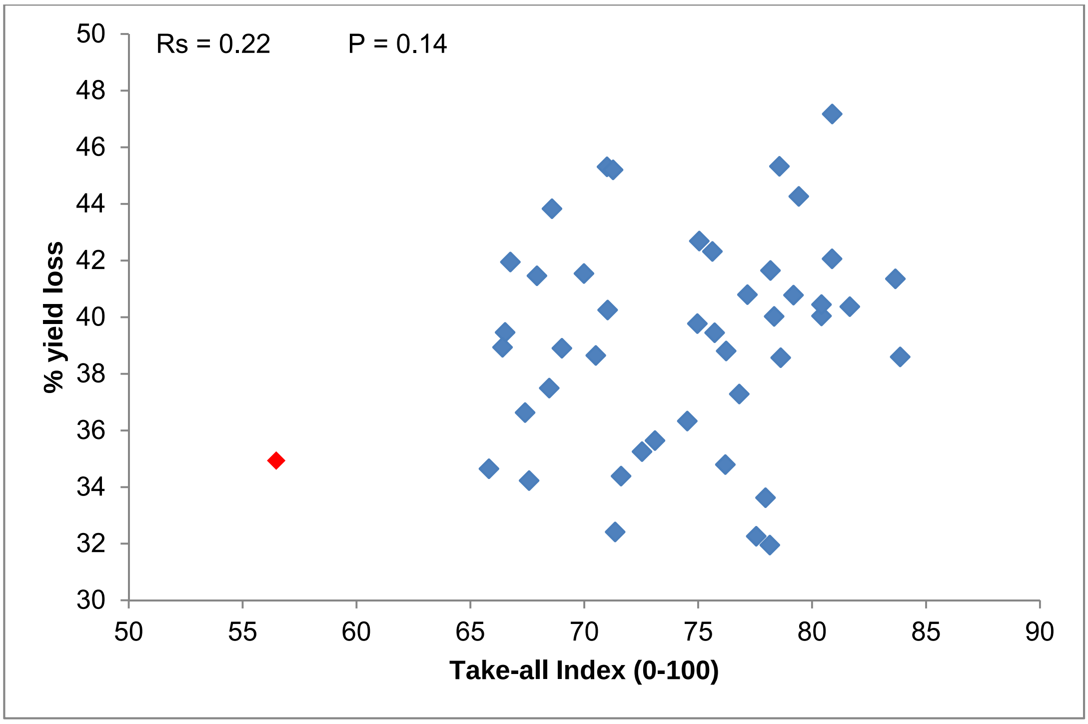


Figure 15. Correlation between the elite winter wheat variety take-all indexes in the 2009 third wheat field trial and average percentage yield loss between the first and third wheat field trials in 2009. Red diamond = winter wheat variety Hereford, with the lowest take-all index out of the varieties tested.

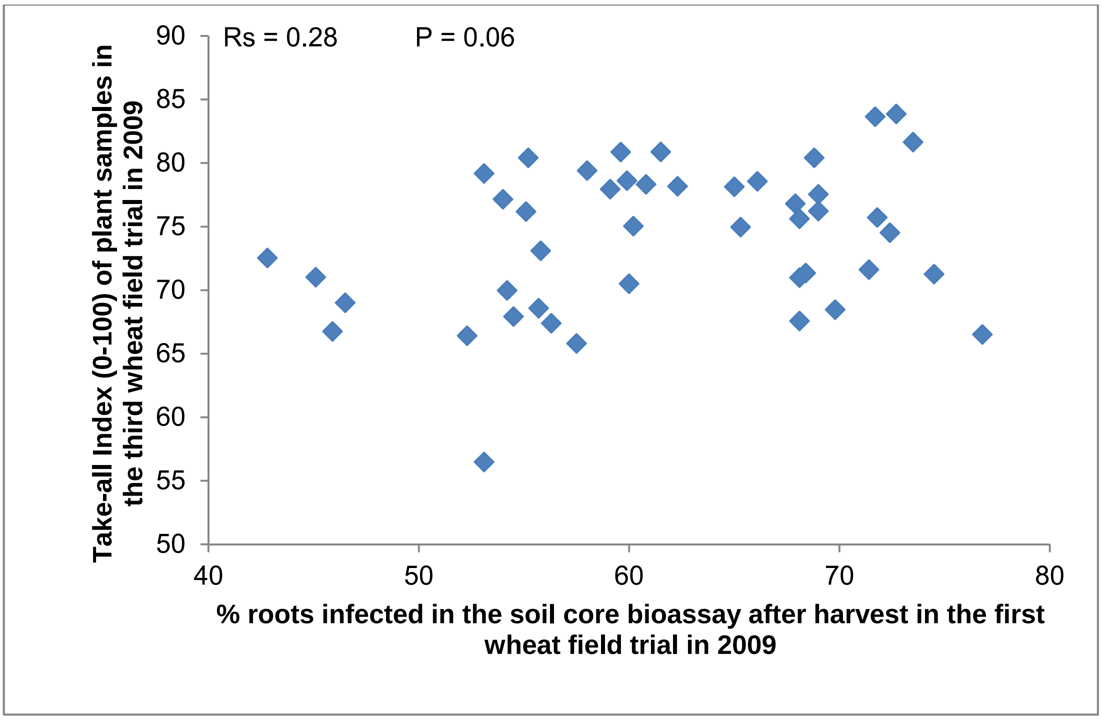


Figure 16. Relationship between the ability of elite wheat varieties to build-up take-all inoculum in the soil during a first wheat crop and their susceptibility to take-all infection in a third wheat high take-all disease pressure trial in 2009.

In 2010 forty-two of the same elite wheat varieties plus 3 replacement varieties (due to limited seed availability of original varieties) were again assessed for their susceptibility to take-all in a third wheat field trial. Unfortunately no seed was available of the variety Hereford, which had the lowest take-all index in the previous 2009 trial. In contrast to the 2009 trial there was a significant effect of variety on take-all disease at the spring sampling point (Percentage plants infected, $P < 0.001$; number of take-all infected roots per plant, $P < 0.001$). The wheat variety Hereward had the highest incidence of take-all (63.8% plants infected) (Figure 17). The incidence (% plants infected) and severity (number of take-all infected roots per plant) of take-all disease were highly correlated ($Rs = 0.95$, $P < 0.001$, $n = 45$).

At the summer sampling point there was an average of 77.3% of plants infected and an average take-all index of 21.56 (compared to an average take-all index of 74.07 in the 2009 trial). This lower disease pressure is probably due to the dry weather in spring 2010 which is unfavourable for take-all development. At the summer sampling point in 2010, at this lower disease pressure, there was now no significant effect of variety detected (Percentage plants infected, $P = 0.496$; Take-all Index, $P = 0.169$). There were also no significant correlations between the amount of take-all disease in the spring and summer sampling points in 2010.

There was an average yield of 8.65 tonnes/ha in this third wheat trial. By comparison there was an average yield of 9.80 tonnes/ha for the same 45 varieties x 4 reps in the first wheat trial in this year (in the absence of take-all root infection). The average percentage yield loss between the 1st and 3rd wheat field trials was 11.79% (compared with a yield loss of 39.15% for the same trials in 2009). As in 2009 there was a significant relationship between variety yields in the 2010 first and third wheat field trials ($Rs = 0.68$, $P < 0.001$, $n = 45$). Varietal percentage yield loss was not correlated with take-all incidence or severity in the spring (% plants infected in the spring and % yield loss, $Rs = -0.01$, $P = 0.92$, $n = 45$; number of take-all infected roots per plant in the spring and % yield loss, $Rs = -0.05$, $P = 0.73$, $n = 45$), or with the take-all index in the summer ($Rs = -0.03$, $P = 0.82$, $n = 45$).

As in the 2009 elite wheat field trials there was also no significant correlation between the ability of varieties to build-up inoculum of the take-all fungus in the 2010 first wheat field trial and their susceptibility to take-all in the 2010 third wheat field trial ($Rs = 0.12$, $P = 0.44$, $n = 45$).

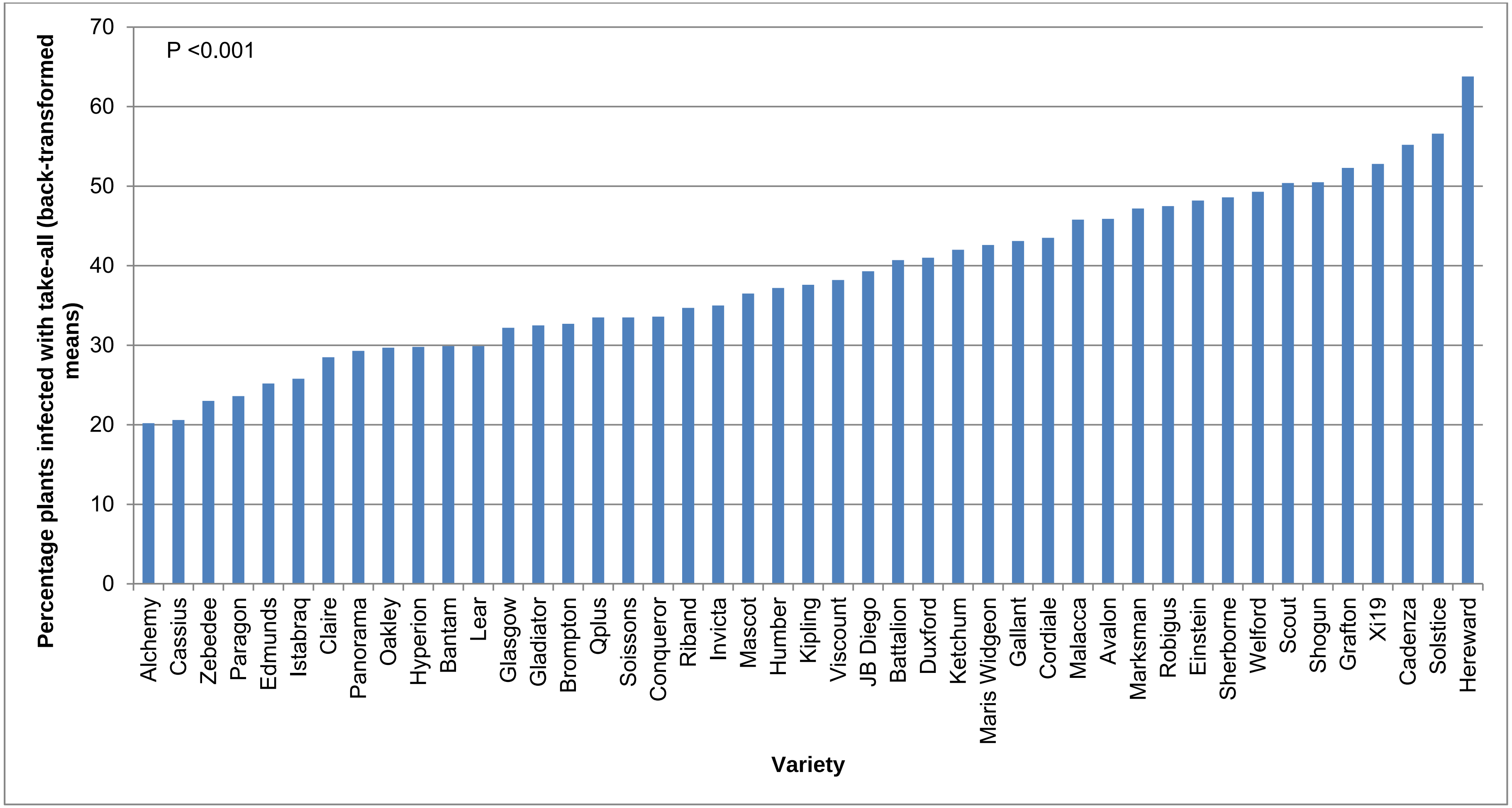


Figure 17. Incidence of take-all disease in the spring of the 2010 elite winter wheat variety trial sown as a third wheat crop.

In 2011 the number of elite wheat varieties included in the 3rd wheat field trial was reduced to 10 based on the limited differences between varieties in the two previous years. Eight varieties were chosen to give a range of possible susceptibilities to take-all. This included the winter wheat variety Hereford, which had the lowest take-all index in the 2009 field trial and also Hereward, which was one of the most severely infected varieties in 2009 and 2010. Two new Recommended List varieties were also included, Kingdom (Syngenta Seeds) and KWS Stirling (KWS UK Ltd).

In the spring an average of 52.1% plants were infected with take-all and there were 0.82 take-all infected roots per plant. There was no significant effect of variety on either disease variable (Percentage plants infected with take-all, P = 0.813; Number of take-all infected roots per plant, P = 0.809). At the summer sampling point there was now a significant effect of variety on the severity of take-all disease (P = 0.019) (Figure 18). The variety Hereford had the lowest take-all index (24.7), with an average across all 10 varieties of 41.6. As in 2009 and 2010 there were no significant correlations between take-all disease in the spring and summer (Spearman's rank correlation analysis, data not shown).

There was an average grain yield of 8.59 tonnes/ha in this 2011 third wheat field trial. Eight of the ten varieties (excluding Hereford and KWS Stirling) were also included in the 2011 first wheat field trial. The average yield of these varieties sown in the first wheat trial was 11.86 tonnes/ha. There was no significant correlation between percentage yield loss and the percentage of plants infected with take-all (Rs = 0.52, P = 0.16, n = 8) or the take-all index (Rs = 0.56, P = 0.12, n = 8) in the summer. The 2011 first wheat field trial was compromised by the presence of the competing fungus *Phialophora graminicola* and a carry-over of take-all inoculum before the trial leading to higher amounts of take-all root infection than expected in certain parts of the trial (see section 3.1). It was therefore not possible to compare take-all incidence and severity of the ten varieties in the third wheat trial with their first wheat inoculum build-up performance in 2011 as inoculum build-up was highly variable across the first wheat trial site.

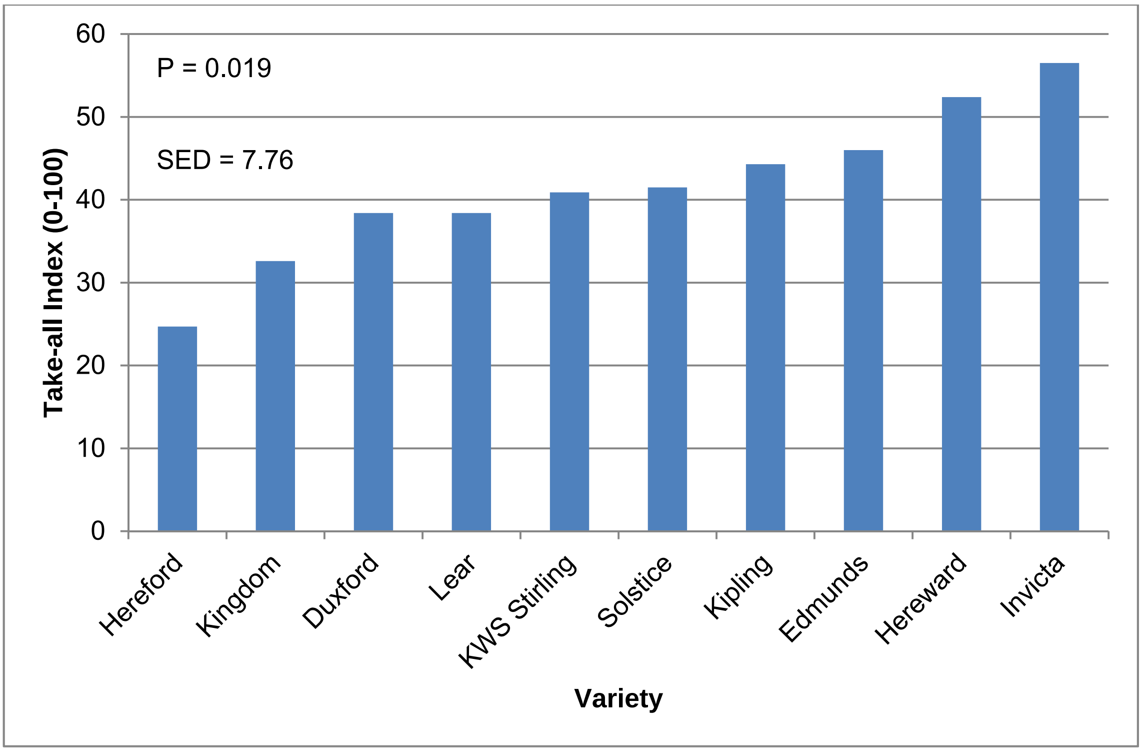


Figure 18. Take-all disease severity in the summer of the 2011 elite winter wheat variety trial sown as a third wheat crop.

After all three field trials were completed a combined year analysis of the spring and summer disease variables was carried out using a REML analysis. However, in general the results from the combined year analysis are dominated by the first year of results in 2009. This is because there was a lower level of residual variance in the first year trial than years 2 and 3. The means are formed from weighted combinations from the different years, the weights being inversely proportional to the size of the variability.

Significant differences between varieties were detected in both the spring and summer combined year analysis, suggesting that modern hexaploid wheat varieties differ in their susceptibility to take-all disease. However, in the individual year analyses as discussed above, the susceptibility of varieties to take-all was not generally related to percentage yield loss. In the combined analysis all of the varieties, except Hereford and Kingdom, had over 90% plants infected with take-all at the summer sampling point. These two varieties also had the lowest take-all index (Figure 19). Hereford was included in two trial years (2009 and 2010) and Kingdom was only in the final trial year (2011).

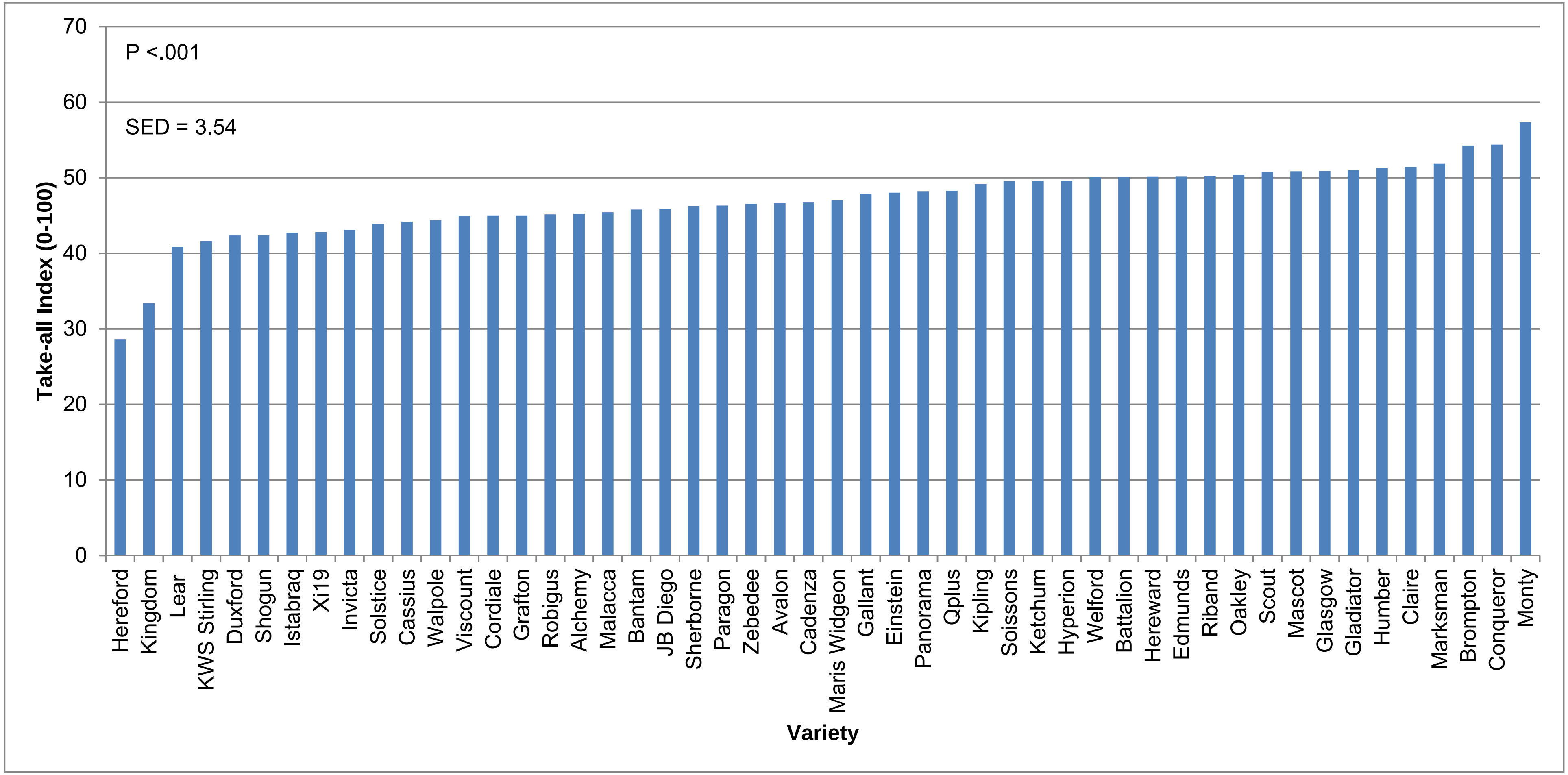


Figure 19. Combined year analysis: Take-all disease on fifty winter wheat varieties in the summer of three third wheat field trials in the harvest years 2009, 2010 and 2011.

5. Discussion

The main purpose of the PhD project was to identify sources of genetic resistance to the take-all fungus, *Gaeumannomyces graminis* var. *tritici*, which could be used to improve the resistance of hexaploid wheat. The research focussed on two main areas: the ability of wheat varieties to build-up take-all inoculum during a first wheat crop and the susceptibility of wheat varieties to take-all root infection in third wheat field trials.

In the 2009 first wheat field trial significant differences between varieties were detected in their take-all inoculum building abilities. Nine of the 45 varieties tested built up less inoculum in the soil than the previously identified low TAB variety Cadenza. Unfortunately variety performance could not be confirmed in 2010 and 2011. In 2010 the exceptionally dry weather in the spring and summer resulted in a failure of take-all inoculum to develop across the whole trial site (and in other trials across the Rothamsted farm). The following year background variation in the 2011 trial site was unusually high, masking the effect of variety. This illustrates some of the problems associated with take-all field research. Take-all is known to be a notoriously ‘patchy’ disease (Hornby *et al.*, 1998) and this makes it a challenge to detect treatment effects. Field trials are also vulnerable to environmental variation. However, take-all inoculum build-up cannot be accurately reproduced in glasshouse studies (R.J. Gutteridge, unpublished data) so is dependent on experimentation under field conditions.

The presence of significant populations of the fungus *Phialophora graminicola* in the 2011 first wheat field trial site and in the first rotation trial worked to restrict the build-up of take-all inoculum. *P. graminicola* is related to *Ggt* but is confined to the epidermis and root cortex and is only very weakly pathogenic (Hornby *et al.*, 1998). Early work by Deacon (1973) and Slope et al. (1979) described the control of take-all due to *Phialophora graminicola* which had developed in grass leys over two or more years prior to wheat being sown. There is no information on how common naturally occurring populations of *Phialophora* spp. are in the UK. On the Rothamsted farm 4 out of approx. 40 fields have now been found to contain populations of *Phialophora* spp. and so are not suitable for take-all trials. We do not know if these are permanent resident populations or if in the future these fields could be used again for take-all trials. The presence of *P. graminicola* across the trial site makes it more difficult to interpret results as this fungus can act to delay the progress of take-all epidemics. It is also not known if there are differences between varieties in their ability to act as hosts to this fungus. To prevent the 2011 situation field testing is now carried out to help select new fields suitable for trials.

Despite these difficulties the WGIN study (McMillan *et al.*, 2011) and 2009 elite winter wheat field trial demonstrate that important differences exist between elite wheat varieties in their ability to build-up take-all inoculum in the soil. The strong influence of environmental conditions and the time consuming and labour intensive nature of the field trials used to assess the TAB trait are a significant problem for screening for the TAB trait in wheat breeding programmes. The situation could be improved if tightly linked markers were developed for the TAB trait allowing initial selection of lines based on this genetic information. Then the time-consuming phenotyping would occur only in the later generations when fewer lines remain. The genetic basis of the trait is now being explored within the on-going WGIN programme.

The practical significance of varietal differences in TAB was investigated in rotational trials. I was involved in the first two of these trials (carried out during my PhD) and results have been reported here. The aim of the trials was to explore take-all disease levels and yields in second wheats after the low TAB Cadenza and high TAB Hereward wheat varieties. This would generate information on whether selection of a low TAB variety is a practical disease management strategy for controlling take-all where a farmer wishes to grow consecutive wheat crops. Conditions in the first year of both trials were not ideal; the presence of *Phialophora graminicola* in the first rotation trial and the very dry weather in the second rotation trial both worked to restrict inoculum build-up. Despite this the two trials do provide evidence that growing a low TAB first wheat variety does reduce take-all disease in the following second wheat crop and improve yields, even in years generally unfavourable for take-all development. There were no significant interactions between first wheat variety and second wheat variety treatments in both trials. This is important as it means that growing a low TAB first wheat variety should be of benefit regardless of the following second wheat variety, so does not impose restrictions on second wheat variety choice.

The underlying mechanism(s) influencing take-all inoculum build-up in the field are not known. The soil core bioassay measures the take-all infectivity of the soil and in the absence of a direct method to quantify take-all inoculum the bioassay method has been used over many years as a gauge of the amount of take-all inoculum in the soil capable of causing visible root disease. However, the infectivity of the soil in the bioassay could also be influenced by other factors, including the soil physical and chemical environment, microbial community and the pathogenicity of take-all isolates present. A molecular method has been developed in Australia that is capable of quantifying the amount of take-all DNA in soil samples (Ophel-Keller *et al.*, 2008). Studies comparing the bioassay and molecular method have shown that in general there is a good correlation between the amount of take-all DNA measured in the soil and the infectivity of the soil measured using the soil core bioassay. This suggests that in general it is the actual amount of take-all inoculum in the soil that changes during the first wheat crop, not just the infectivity of existing take-all inoculum in the soil. In the inoculum build-up WGIN study we discussed how varietal differences in inoculum build-up could be influenced by the susceptibility of wheat varieties to take-all root infection, the physical structure of wheat roots and the soil, the microbial communities under different wheat varieties, wheat root exudates, nutrient utilization of wheat plants and crop senescence (McMillan et al., 2011).

The susceptibility of elite wheat varieties to take-all root infection was investigated in the third wheat field trials. In common with previous literature, a range of susceptibilities of hexaploid wheat varieties to take-all were found but differences were generally small and not very consistent between sites and years. This is in contrast to the TAB trait where consistent differences between varieties have been demonstrated within the WGIN trials (McMillan *et al.*, 2011). In this study the performance of the elite varieties in the first and third wheat trials was not closely associated suggesting that the TAB trait is not related to the susceptibility of wheat varieties to take-all root infection. Take-all intensity in the 2009 third wheat trial was generally high for all varieties, and although there were significant differences between varieties, none of the varieties were highly resistant. In 2011, a moderate disease pressure year, there was a bigger difference between varieties, with some varieties having more than double the take-all index of others. However it was not clear whether these differences would result in a significant improvement in yields for the least susceptible varieties. In both 2009 and 2011 the winter wheat variety Hereford stood out as displaying some potential partial resistance to take-all disease (Hereford was not included in 2010 trial due to limited seed availability).

6. Conclusion

This study has demonstrated that important genetic interactions do exist between the take-all fungus and hexaploid wheat which could be utilised to improve the performance of second wheat crops.

The low TAB trait was detected in a range of elite wheat varieties, of different genetic backgrounds. Rotation trials demonstrated the potential benefit of the low TAB phenotype in a first wheat on take-all severity and yields in a second wheat crop. This suggests that farmers could already reduce the risk of take-all in their second wheat crops by appropriate varietal choice from within the currently available wheat varieties. However, when environmental conditions are highly favourable a significant amount of inoculum can build-up under even low building varieties. Appropriate choice of a low TAB variety to grow as a first wheat is not therefore expected to negate completely the risk of take-all in the following crop, but would instead be used in conjunction with other control measures as part of an integrated approach. Some potential partial resistance to take-all was also detected within hexaploid wheat for the variety Hereford. If this resistance proves consistent, over sites and seasons, and the genetic basis of the trait could be identified and incorporated into breeding lines it could provide a way for farmers to further limit their risk of severe take-all when sowing a second wheat crop. The candidate variety Hereford was found to be very susceptible to brown rust and therefore did not make the HGCA Recommended List.

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