

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

Submitted by

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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 but plant material that would be useful for take-all control via a genetic approach has not been identified 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 in hexaploid wheat and a related diploid wheat species, *Triticum monococcum*. 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, and pedigree and molecular marker analyses were carried out to investigate the genetic sources of the TAB trait. 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 fifty elite hexaploid wheat varieties and thirty-four *T. monococcum* accessions to take-all was evaluated in third wheat field trials. Both *T. aestivum* (variety Hereford) and *T. monococcum* (MDR031 and MDR046) genotypes with some partial resistance to take-all were identified. A seedling pot test method as a screen for resistance was also explored but the results were found not to be closely related to the susceptibility of adult plants in field trials. The implications of these new findings for the control of take-all and further research are discussed.

Table of Contents	
Acknowledgements	2
Abstract	3
List of Figures	8
List of Tables	12
CHAPTER 1: INTRODUCTION	17
1.1. Soil-borne plant pathogens	19
1.2. Take-all disease of wheat	19
1.3. UK wheat varieties	22
1.4. The <i>Gaeumannomyces</i> genus	23
1.5. Take-all infection process and epidemiology	24
1.6. Take-all control methods	27
1.6.1. <i>Cultural control</i>	27
1.6.2. <i>Biological control</i>	29
<i>Take-all decline (TAD)</i>	29
<i>Phialophora spp.</i>	30
<i>Introduced biological control agents</i>	32
1.6.3. <i>Chemical control</i>	33
1.7. Genetic control	34
1.7.1. <i>Resistance of oats to take-all</i>	35
1.7.2. <i>Resistance of rye to take-all</i>	36
1.7.3. <i>Resistance of other grass species</i>	37
1.7.4. <i>Resistance of wheat (<i>Triticum aestivum</i>) to take-all</i>	38
1.7.5. <i>Difficulties assessing wheat germplasm for resistance to take-all</i>	42
1.8. Aims and Objectives.....	44
CHAPTER 2: GENERAL MATERIALS AND METHODS	45
2.1. Maintenance of <i>Ggt</i> isolates and long term storage	45
2.2. Field trials	45
2.2.1. <i>Soil core bioassay</i>	46
2.2.2. <i>Plant sampling and take-all disease assessment</i>	47
<i>Spring</i>	47
<i>Summer</i>	47
2.3. Pot test method	48
2.3.1. <i>Preparing Ggt inoculum</i>	48
2.3.2. <i>The pot test method</i>	49

2.3.3. <i>Soil calibration</i>	49
2.4. Statistics.....	49
CHAPTER 3: FIELD EVALUATION OF THE TAKE-ALL INOCULUM BUILD-UP (TAB) TRAIT	51
3.1. Introduction	51
3.2. Materials and Methods	54
3.2.1. <i>Elite winter wheat varieties and inoculum build-up 2009-2011</i>	56
3.2.2. <i>Rotation experiments</i>	64
3.2.3. <i>Microscopic analysis</i>	68
3.3. Results	68
3.3.1. <i>Elite winter wheat varieties and inoculum build-up 2009-2011</i>	68
3.3.2. <i>Epidemiology studies 2009-2011</i>	79
3.3.3. <i>Take-all inoculum build-up 2004-2011</i>	89
3.3.4. <i>Rotation experiments</i>	92
3.4. Discussion.....	103
CHAPTER 4: GENETIC EVALUATION OF THE TAB TRAIT	114
4.1. Introduction	114
4.2. Materials and Methods	117
4.2.1. <i>Pedigree analysis</i>	117
4.2.2. <i>SSR analyses</i>	118
<i>DNA extraction</i>	118
<i>Simple Sequence Repeat (SSR) analyses</i>	119
4.3. Results	120
4.3.1. <i>Sources of the TAB trait in elite winter wheat varieties: pedigree analysis</i> ...	120
4.3.2. <i>Investigation of the genetic basis of the TAB trait: SSR marker analyses</i>	128
4.4. Discussion.....	139
CHAPTER 5: CHARACTERISATION OF A NEW GGT ISOLATE COLLECTION	141
5.1. Introduction	141
5.2. Materials and Methods	143
5.2.1. <i>Ggt isolations</i>	143
5.2.2. <i>DNA extraction and PCR using Ggt specific primers</i>	143
5.2.3. <i>Fungicide sensitivity testing</i>	146
5.2.4. <i>Pathogenicity assay on wheat</i>	146
5.3. Results	147

5.3.1. Isolate characterisation by Ggt-specific PCR	147
5.3.2. Isolate characterisation by sensitivity to the fungicide silthiofam in vitro	150
5.3.3. Relationship between genetic sub-population type and silthiofam sensitivity	151
5.3.4. Isolate pathogenicity on wheat	152
5.4. Discussion.....	154
CHAPTER 6: EVALUATION OF ELITE WINTER WHEAT VARIETIES FOR RESISTANCE TO TAKE-ALL	156
6.1. Introduction	156
6.2. Materials and Methods	160
6.2.1. Elite winter wheat field trials.....	160
6.2.2. Elite winter wheat pot tests	167
6.3. Results	168
6.3.1. Response of winter wheat varieties to take-all under field conditions	168
2009.....	168
2010.....	182
2011.....	191
6.3.2. Combined year comparison	198
6.3.3. Response of winter wheat varieties to take-all in seedling pot tests.....	202
6.4. Discussion.....	208
CHAPTER 7: EVALUATION OF OTHER WHEAT GERMPLASM FOR RESISTANCE TO TAKE-ALL	216
7.1. Introduction	216
7.2. Materials and Methods	219
7.2.1. Diploid wheat (<i>T. monococcum</i>) field trials	219
7.2.2. <i>T. monococcum</i> pot tests	225
7.2.3. <i>T. monococcum</i> DArT diversity analysis	229
7.2.4. <i>B. distachyon</i> -take-all pathogenicity test.....	229
7.3. Results	231
7.3.1. Response of <i>T. monococcum</i> to take-all under field conditions	231
7.3.2. Response of <i>T. monococcum</i> to take-all in pot tests	238
7.3.3. <i>T. monococcum</i> DArT diversity analysis	245
7.3.4. Response of <i>B. distachyon</i> ecotypes to take-all	247
7.4. Discussion.....	249
CHAPTER 8: GENERAL DISCUSSION	257
8.1. Project summary	257

8.2. Further work	258
8.3. Using QTL mapping information to develop new wheat varieties	260
8.4. Sequencing the wheat (<i>Triticum aestivum</i>) genome	263
8.5. Sequencing the <i>Ggt</i> genome	264
8.6. Metagenomics.....	266
8.7. Future of take-all control	268
8.8. Food security	269
Appendix 3.1. Elite winter wheat varieties and inoculum build-up field plans	274
Appendix 3.2. Rotation trials 1 and 2 field plans	277
Appendix 3.3. Visual flag leaf senescence scoring	279
Appendix 4.1. Variety numbering in Li-Cor gel analysis	280
Appendix 4.2. Preparation of DNA extraction buffers.....	281
Appendix 4.3. Li-Cor gel preparation.....	282
Appendix 4.4. SSR analyses.....	283
Appendix 5.1. DNA extraction buffer	294
Appendix 5.2. <i>Ggt</i> isolate collection used in PhD study	295
Appendix 6.1. Elite winter wheat varieties and resistance to take-all field plans	296
Appendix 7.1. Diploid wheat and resistance to take-all field plans	299
Abbreviations	304
References	305

List of Figures

Chapter 1

Figure 1.1. Take-all lesions and stem base blackening on wheat seedlings.....	20
Figure 1.2. Take-all patch.....	20
Figure 1.3. Dark runner hyphae of the take-all fungus on the surface of a wheat root and black take-all lesions within the root tissue.....	25

Chapter 3

Figure 3.1. Soil core bioassay method.....	52
Figure 3.2. 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)....	73
Figure 3.3. Per plot correlation between the percentage roots infected on bait plants in the soil core bioassay and the take-all index of plant samples in the 2010 first wheat field trial (10/R/WW/1032).....	74
Figure 3.4. Percentage roots infected in the soil core bioassay per plot in the 2009 elite winter wheat TAB field trial (09/R/WW/916).....	76
Figure 3.5. Percentage roots infected in the soil core bioassay per plot in the 2011 elite winter wheat TAB field trial (11/R/WW/1115).....	77
Figure 3.6. Per plot correlation between the percentage roots infected on bait plants in the soil core bioassay and the take-all index of plant samples in the 2011 first wheat field trial (11/R/WW/1115).....	78
Figure 3.7. Per plot correlation between the percentage roots infected on bait plants in the soil core bioassay and yields in the 2011 first wheat field trial (11/R/WW/1115)...	78
Figure 3.8. Soil moisture (m ³ water/m ³ soil) (blue bars) and maximum soil temperature (°C) (red line) recorded at a depth of 15cm for the 2011 elite winter wheat TAB field experiment 11/R/WW/1115 (Pastures field) from 20 th May until August 11 th 2011.....	87
Figure 3.9. Spearman's rank correlation between average maximum temperature in July and mean TAB in the 2004-2011 first wheat field trials	91
Figure 3.10. Spearman's rank correlation between rainfall in June and mean TAB in the 2004-2011 first wheat field trials.....	92
Figure 3.11. Spearman's rank correlation between rainfall in August and mean TAB in the 2004-2011 first wheat field trials	92

Figure 3.12. Percentage roots infected in the soil core bioassay per plot after harvest of year 1 of Rotation trial 2 (10/R/CS/706).....	99
Figure 3.13. Per plot correlation between the percentage roots infected on bait plants in the soil core bioassay after harvest in year 1 of rotation trial 2 and the take-all index of plant samples in year 2 (10-11/R/CS/706).....	102
Figure 3.14. Per ‘oversow’ variety correlation between the percentage roots infected on bait plants in the soil core bioassay after harvest in year 1 of rotation trial 2 and the take-all index of plant samples in year 2 (10-11/R/CS/706).....	102

Chapter 4

Figure 4.1. QTL analysis of field data from the Double Haploid (DH) lines oversown with Oakley.....	116
Figure 4.2. Genetic maps of chromosomes A and B showing the location (in red) of the mapped QTLs for low take-all inoculum build-up (TAB).....	116
Figure 4.3. Cadenza family tree (modified from a family tree created by Edward Flatman, RAGT).....	123
Figure 4.4. Claire family tree.....	124
Figure 4.5. Avalon family tree (modified from a family tree created by Edward Flatman, RAGT).....	126
Figure 4.6. Robigus family tree.....	127
Figure 4.7. Marker alleles at XM001, XM002, XM003 and XM004 loci on chromosome A.....	129
Figure 4.8. Marker alleles at XM005, XM006, XM007, XM008 and XM009 loci on chromosome B.....	131

Chapter 5

Figure 5.1. <i>Ggt</i> specific PCR analysis of 20 new <i>Ggt</i> isolates (BC01-BC20) obtained from root isolations of soil bioassay plants using primers Ggtfwd, GgtArev and GgtBrev2 to identify A and B <i>Ggt</i> subpopulations.....	148
Figure 5.2. <i>Ggt</i> specific PCR analysis of 20 new <i>Ggt</i> isolates (BC20-BC40) obtained from root isolations of soil bioassay plants using primers Ggtfwd, GgtArev and GgtBrev2 to identify A and B <i>Ggt</i> subpopulations.....	149
Figure 5.3. Pathogenicity test on wheat seedlings; left: control plant, mock-inoculated, right: <i>Ggt</i> infected seedling with yellowing leaves and stunted growth.....	152

Chapter 6

Figure 6.1. Correlation between the mean varietal take-all patch score and take-all index of plant samples in the 2009 third wheat field trial (09/R/WW/917).....	172
Figure 6.2. Photographs of the first wheat (09/R/WW/916; top picture) and third wheat (09/R/WW/917; bottom picture) variety field trials on the Rothamsted farm.....	173
Figure 6.3. Correlation between the mean varietal take-all patch score and yields in the 2009 third wheat field trial (09/R/WW/917).....	174
Figure 6.4. Correlation between the mean variety take-all index and yields in the 2009 third wheat field trial (09/R/WW/917).....	174
Figure 6.5. Correlation between the elite winter wheat variety take-all patch scores in the 2009 third wheat field trial (09/R/WW/917) and average percentage yield loss between the first (09/R/WW/916) and third wheat field trials.....	175
Figure 6.6. Correlation between the elite winter wheat variety take-all indexes in the 2009 third wheat field trial (09/R/WW/917) and average percentage yield loss between the first (09/R/WW/916) and third wheat field trials.....	176
Figure 6.7. 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.....	177
Figure 6.8. The number of take-all infected seminal roots per plant from April to July for six varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).....	179
Figure 6.9. The number of take-all infected crown roots per plant from April to July for six varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).....	180
Figure 6.10. Correlation between the mean variety take-all index and yields in the 2010 third wheat field trial (10/R/WW/1031).....	185
Figure 6.11. The number of take-all infected seminal roots per plant from April to July for eight varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).....	188
Figure 6.12. The number of take-all infected crown roots per plant from April to July for eight varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).....	188
Figure 6.13. Correlation between the percentage of plants infected with take-all and variety yields in the 2011 third wheat field trial (11/R/WW/1114).....	193

Figure 6.14. Correlation between the take-all index and variety yields in the 2011 third wheat field trial (11/R/WW/1114).....	193
Figure 6.15. The number of take-all infected seminal roots per plant from March to July for two varieties in the 2011 elite winter wheat and resistance to take-all field trial (11/R/WW/1114).....	196
Figure 6.16. The number of take-all infected crown roots per plant from March to July for two varieties in the 2011 elite winter wheat and resistance to take-all field trial (11/R/WW/1114).....	196
Figure 6.17. Correlation between the percentage roots infected of 44 elite winter wheat varieties in pot tests with silthiofam sensitive or B type <i>Ggt</i> isolates.....	207

Chapter 7

Figure 7.1. Principal coordinate analysis of 20 <i>T. monocoicum</i> accessions based on 1041 DArT markers.....	246
Figure 7.2. Dendrogram of genetic similarity among the 20 <i>T. monocoicum</i> accessions constructed based on group average cluster analysis.....	247
Figure 7.3. Pathogenicity test <i>Brachypodium distichon</i> seedlings infected with take-all; left = control plants, right = severely infected seedling with blackened roots, yellowing leaves and stunted growth.....	248

List of Tables

Chapter 3

Table 3.1. Details of the field experiments used to evaluate the TAB trait.....	55
Table 3.2. Winter wheat varieties used in field experiments 09/R/WW/916, 10/R/WW/1032 and 11/R/WW/1115.....	57
Table 3.3. Varieties selected for epidemiology studies in field trials 09/R/WW/916, 10/R/WW/1032 and 11/R/WW/1115.....	60
Table 3.4. Sampling details of the 1 st wheat elite winter wheat variety and take-all inoculum build-up field trials 2009-2011.....	62
Table 3.5. Winter wheat varieties chosen for 2 nd year of rotation experiments 10/R/CS/688 and 11/R/CS/706.....	66
Table 3.6. Sampling details of rotation field trials 2009-2011.....	67
Table 3.7. Take-all infectivity of the soil after harvest of elite winter wheat variety trials sown as first wheat crops, 2009-2011.....	68
Table 3.8. The incidence and severity of take-all disease on plant roots from the first wheat elite winter wheat variety field trials, 2009-2011.....	71
Table 3.9a. Epidemiology study on take-all inoculum build-up from April through to harvest under six winter wheat varieties in the 2009 elite winter wheat TAB field trial (09/R/WW/916).....	81
Table 3.9b. Main effect of variety and month on take-all inoculum build-up in the 2009 epidemiology study (09/R/WW/916).....	82
Table 3.10a. Epidemiology study on take-all inoculum build-up from March through to harvest under six winter wheat varieties in the 2010 elite winter wheat TAB field trial (10/R/WW/1032).....	83
Table 3.10b. Main effect of variety and month on take-all inoculum build-up in the 2010 epidemiology study (10/R/WW/1032).....	84
Table 3.11a. Epidemiology study on take-all inoculum build-up from March through to harvest under six winter wheat varieties in the 2011 elite winter wheat TAB field trial (11/R/WW/1115).....	85
Table 3.11b. Main effect of variety and month on take-all inoculum build-up in the 2011 epidemiology study (11/R/WW/1115).....	86
Table 3.12a. Epidemiology study on soil pH under winter wheat varieties Hereward and Cadenza in the 2011 elite winter wheat and take-all inoculum build-up field trial (11/R/WW/1115).....	88

Table 3.12b. Main effect of variety and month on soil pH in the 2011 elite winter wheat and take-all inoculum build-up field trial (11/R/WW/1115).....	88
Table 3.13. Mean level of inoculum build-up, measured on bait wheat plants in the soil core bioassay, after harvest of first wheat field trials at Rothamsted Research from 2004 to 2011.....	89
Table 3.14. Monthly rainfall (mm) and average maximum temperatures (°C) recorded at Rothamsted from March to August for the field seasons from 2004 to 2011.....	90
Table 3.15. Spearman’s rank correlation between the monthly rainfall (mm) and average maximum temperatures (°C) recorded at Rothamsted from March to August and the mean TAB after harvest in the 2004-2011 first wheat field trials.....	91
Table 3.16. Rotation trial 1 (Year 1: 09/R/CS/688; Year 2: 10/R/CS/688). Take-all infectivity of the soil after the first wheat source varieties Cadenza and Hereward, and take-all disease and yield data in the subsequent second wheat oversow.....	94
Table 3.17. Rotation trial 1 (Year 1: 09/R/CS/688; Year 2: 10/R/CS/688). Take-all infectivity of the soil after harvest of year 1 plots, analysed by the plot locations of the 2 nd wheat oversow varieties. Take-all disease and yield data in the subsequent second wheat oversow varieties.....	95
Table 3.18. Rotation trial 2 (Year 1: 10/R/CS/706; Year 2: 11/R/CS/706). Take-all infectivity of the soil after the first wheat source varieties Cadenza and Hereward, and take-all disease and yield data in the subsequent second wheat oversow.....	100
Table 3.19. Rotation trial 2 (Year 1: 10/R/CS/706; Year 2: 11/R/CS/706). Take-all infectivity of the soil after harvest of year 1 plots, analysed by the plot locations of the 2 nd wheat oversow varieties. Take-all disease and yield data in the subsequent second wheat oversow varieties.....	101
Table 3.20. Winter wheat varieties chosen for 2012 elite winter wheat and take-all inoculum build-up trial (12/R/WW/1211).....	108

Chapter 4

Table 4.1. Pedigree information of varieties in the SSR marker analysis.....	120
Table 4.2. Inoculum building ability and pedigree analysis of the elite winter wheat varieties in the 2009 PhD field trial.....	128
Table 4.3. Inoculum building ability and marker allele comparison between the low TAB variety Cadenza and other elite winter wheat varieties.....	134
Table 4.4. Inoculum building ability and marker allele comparison between the high TAB variety Avalon and other elite winter wheat varieties.....	135

Table 4.5. Microsatellite allele comparison between the mapping population parent varieties, Avalon and Cadenza, and other elite winter wheat varieties used in the PhD study.....	136
---	------------

Chapter 5

Table 5.1. PCR primer sequences and PCR conditions for identification of A/B type <i>Ggt</i> isolates.....	145
---	------------

Table 5.2. Sensitivity of isolates to the fungicide silthiofam as tested <i>in vitro</i> on silthiofam amended PDA plates.....	150
---	------------

Table 5.3. Number of <i>Ggt</i> isolates from Bones Close culture collection that were classified as A or B type according to ITS2 diversity and sensitive or resistant to the fungicide silthiofam in <i>in vitro</i> fungicide sensitivity tests.....	151
--	------------

Table 5.4. Pathogenicity of <i>Ggt</i> isolates with silthiofam treated wheat seed and untreated seed.....	153
---	------------

Chapter 6

Table 6.1. Details of the field experiments used to evaluate the susceptibility to take-all of elite winter wheat varieties.....	162
---	------------

Table 6.2. Elite winter wheat varieties used in field experiments 09/R/WW/917, 10/R/WW/1031 and 11/R/WW/1114.....	162
--	------------

Table 6.3. Varieties selected for epidemiology studies in field trials 09/R/WW/917, 10/R/WW/1031 and 11/R/WW/1114.....	165
---	------------

Table 6.4. Sampling details of the 3 rd wheat elite winter wheat variety and susceptibility to take-all field trials 2009-2011.....	166
---	------------

Table 6.5. Take-all disease in the spring and summer of the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).....	169
--	------------

Table 6.6a. Take-all incidence from April to July for six varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).....	178
---	------------

Table 6.6b. Take-all incidence from April to July for six varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).....	179
---	------------

Table 6.7. Seminal root development from April to July for six winter wheat varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917)...	181
---	------------

Table 6.8. Crown root development from April to July for six winter wheat varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).....	181
---	------------

Table 6.9. Mean number of seminal and crown roots per plant in July for six winter wheat varieties in the 2009 1 st wheat field trial (09/R/WW/916).....	182
Table 6.10. Take-all disease in the spring and summer of the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).....	183
Table 6.11a. Take-all incidence from April to July for eight varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).....	187
Table 6.11b. Take-all incidence from April to July for eight varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).....	187
Table 6.12. Seminal root development from April to July for eight winter wheat varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).....	189
Table 6.13. Crown root development from April to July for eight winter wheat varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031)..	190
Table 6.14. Mean number of seminal and crown roots per plant in July for eight winter wheat varieties in the 2010 1 st wheat field trial (10/R/WW/1032).....	190
Table 6.15. Take-all disease in the spring and summer of the 2011 elite winter wheat and resistance to take-all field trial (11/R/WW/1114).....	192
Table 6.16a. Take-all incidence from March to July for two varieties in the 2011 elite winter wheat and resistance to take-all field trial (11/R/WW/1114).....	195
Table 6.16b. Take-all incidence from March to July for two varieties in the 2011 elite winter wheat and resistance to take-all field trial (11/R/WW/1114).....	195
Table 6.17. Seminal root development from March to July for the wheat varieties Hereford and Hereward in the 2011 elite winter wheat and resistance to take-all 3 rd wheat field trial (11R/WW/1114).....	197
Table 6.18. Crown root development from March to July for the wheat varieties Hereford and Hereward in the 2011 elite winter wheat and resistance to take-all 3 rd wheat field trial (11R/WW/1114).....	197
Table 6.19. Combined year analysis of take-all disease of fifty winter wheat varieties in the spring and summer of three third wheat field trials (09/R/WW/917, 10/R/WW/1031, 11/R/WW/1114).....	199
Table 6.20. Susceptibility of elite winter wheat varieties to take-all infection in a seedling pot test using silthiofam resistant (R) <i>Ggt</i> isolates.....	203
Table 6.21. Susceptibility of elite winter wheat varieties to take-all infection in a seedling pot test using silthiofam sensitive (S) <i>Ggt</i> isolates.....	204

Table 6.22. Susceptibility of elite winter wheat varieties to take-all infection in a seedling pot test using B type <i>Ggt</i> isolates based on molecular classification.....	206
Table 6.23. Spearman’s rank correlation between susceptibility of wheat varieties in five week pot tests and susceptibility in the field.....	208

Chapter 7

Table 7.1. <i>T. monococcum</i> accessions used in the diploid wheat take-all resistance study field trials 2006 and 2008-2011.....	222
Table 7.2. Details of the diploid wheat and take-all resistance field trials.....	224
Table 7.3. <i>T. monococcum</i> accessions screened in the 2007 diploid wheat pot tests 1 and 3.....	226
Table 7.4. <i>Brachypodium distachyon</i> ecotypes used in take-all pathogenicity study..	230
Table 7.5a. Take-all index of control cereal species, <i>T. monococcum</i> accessions, tetraploid wheat varieties and <i>Ae. speltoides</i> in the diploid wheat and take-all resistance field trials 2006-2011.....	232
Table 7.5b. Take-all index of control cereal species and hexaploid wheat varieties in the diploid wheat and take-all resistance field trials 2006-2011.....	234
Table 7.6. Combined year analysis of the take-all index of <i>T. monococcum</i> accessions, hexaploid and tetraploid wheat varieties in five years of field experiments (2006, 2008-2011).....	236
Table 7.7. Susceptibility of <i>T. monococcum</i> and modern hexaploid wheat varieties to take-all infection in a seedling pot test 2006.....	239
Table 7.8. Susceptibility of <i>T. monococcum</i> to take-all infection in seedling pot test 1 2007.....	240
Table 7.9. Susceptibility of <i>T. monococcum</i> to take-all infection in seedling pot test 3 2007.....	242
Table 7.10. Susceptibility of <i>T. monococcum</i> accessions to take-all infection in a seedling pot test in 2012.....	244
Table 7.11. Pathogenicity of silthiofam sensitive (S) and resistant (R) <i>Ggt</i> isolates on five <i>Brachypodium distachyon</i> ecotypes.....	248
Table 7.12. Summary of susceptibility of <i>T. monococcum</i> accessions to take-all in field trials at Rothamsted 2006 & 2008-2011.....	251

CHAPTER 1: INTRODUCTION

Plant diseases have had a huge effect on crop productivity and human history. Flood (2010) describes the Irish Potato Famine, starting in 1845, as the classic example of the effect of plant disease on human history; an over reliance on potatoes as the main food crop combined with a series of failed harvests due to potato blight (caused by the pathogen *Phytophthora infestans*) led to the death or emigration of over 2 million people in Ireland. In the 20th century the spread of wheat stripe rust (caused by the pathogen *Puccinia striiformis* f. sp. *graminis*) throughout the United States seriously affected wheat production causing large economic losses for farmers (Carleton, 1915, Chen, 2007). Crop diseases are also considered a risk to current and future global food security (Strange & Scott, 2005, Mahmuti et al., 2009, Flood, 2010, Cook et al., 2011), due to the large reduction in attainable yields worldwide.

Wheat (*Triticum* species) is grown worldwide and is the dominant cereal crop in the Northern hemisphere (Oerke, 2006). In 2010 world wheat production was over 650 million tonnes (Food and Agriculture Organisation of the United Nations, FAOSTAT, <http://faostat.fao.org/>). The most widely grown domesticated wheat is hexaploid bread wheat *Triticum aestivum*. *Triticum aestivum* was derived from hybridisation between tetraploid wheat (containing the A and B genomes) with a wild diploid Goat grass (*Aegilops tauschii*, syn *Aegilops squarrosa*). *Aegilops tauschii* contributed the D genome to modern bread wheat so that it contains 6 sets of chromosomes (AABBDD)(Hancock, 2004, Dvorak et al., 2012). This allopolyploid genome has a total of 21 chromosome pairs. Wheat was first domesticated ~10,000 years ago and commercial wheat varieties in the UK have been developed during the twentieth century from intensive wheat breeding programmes to select for higher yields and desirable grain quality and crop performance traits. Despite this, substantial financial losses are attributable to various abiotic stress as well as pest and diseases of wheat. On average pests and pathogens, including weeds, insects, animal pests, pathogens and viruses cause losses in wheat of up to 40% worldwide (Oerke, 2006). This figure for Northwest Europe is 14%. From a global perspective, the biotic stresses caused by weeds are the most important problem of wheat. However, in temperate regions fungal diseases tend to have a greater impact as the intensity of cropping increases. Plant diseases are more common in cultivated crops than in natural wild plant communities (Stuthman et al., 2007, Stukenbrock & McDonald, 2008), and increases in field sizes, host plant densities

and crop species genetic uniformity are all thought to contribute to the increase in plant disease epidemics as agriculture has intensified (Zadoks, 1993).

There are six major types of pathogens which have evolved to cause plant diseases: viruses, bacteria, fungi, oomycetes, nematodes and parasitic plants. The most important diseases of wheat worldwide are caused by fungal pathogens including the diseases stem rust, leaf rust, powdery mildew, leaf blotch and *Rhizoctonia* root rot (Strange & Scott, 2005). Barley yellow dwarf virus is the principal viral disease of wheat, causing widespread stunting of plants and yield losses (Miller et al., 2002). The most important bacterial disease is bacterial leaf streak caused by *Xanthomonas translucens* pv. *undulosa* (Strange & Scott, 2005). In the UK, Septoria leaf blotch caused by the fungal pathogen *Mycosphaerella graminicola* is probably the most important foliar disease of wheat (Hardwick et al., 2001, Bearchell et al., 2005). Other important diseases in the UK and northwest Europe include the foliar diseases brown rust, yellow rust and powdery mildew, and the stem base disease eyespot (Loyce et al., 2008).

Plant pathogens can be classified based on their host range as specialists or generalists, and/or based on the specific plant tissues that they are able to infect (Barrett & Heil, 2012) and/or on their lifestyle as biotrophs or necrotrophs (Oliver & Ipcho, 2004, Glazebrook, 2005), depending on whether they obtain nutrients from living or dead host tissue. There is also a third grouping based on lifestyle, this is the hemibiotrophs, for pathogens where the initial infection involves biotrophic growth and then switches to necrotrophic growth (Rohel et al., 2001). For example *Mycosphaerella graminicola*, the causal agent of Septoria leaf blotch of wheat, is a hemibiotroph, having an initial biotrophic phase upon infection before switching to necrotrophic growth (Rohel et al., 2001, Keon et al., 2007). *Mycosphaerella graminicola* is thought to have emerged during wheat domestication (Stukenbrock et al., 2007) and exclusively infects wheat (Eyal et al., 1973). In contrast *Puccinia graminis*, the stem rust pathogen, is an obligate biotroph, found as formae speciales infecting individual hosts (Staples, 2000).

To be able to effectively control plant diseases it is important to understand how pathogens may evolve and the nature of host-pathogen interactions. One of the best control strategies is the use of genetically disease resistant host plants. Strange and Scott (2005) suggest that humans have probably selected disease resistant plants since the beginning of crop domestication and cultivation, over 10,000 years ago.

1.1. Soil-borne plant pathogens

Plant root and stem base diseases are predominantly caused by soil-borne fungal or oomycete pathogen species (Anees et al., 2010). Most are necrotrophic pathogens with wide host ranges (Raaijmakers et al., 2009). Common soil-borne pathogens include *Fusarium* spp. (Alabouvette et al., 2009, Chandra et al., 2011), *Pythium* spp. (Martin & Loper, 1999), *Phytophthora* spp. (Huitema et al., 2004), *Rhizoctonia solani* (Vilgalys & Cubeta, 1994), *Gaeumannomyces graminis* (Walker, 1981), and the eyespot pathogens *Oculimacula yallundae* and *O. acuformis* (Crous et al., 2003, Sheng et al., 2012). Soil-borne microorganisms can also act as vectors for viral diseases, for example the fungus *Polymyxa betae* acts as a vector for beet necrotic yellow vein virus, causing rhizomania root disease in sugarbeet (McGrann et al., 2009). The soil-borne fungus *Fusarium oxysporum* infects the roots of a wide range of host species and was recently ranked 5th out of the top 10 most scientifically and economically important fungal plant pathogens in the journal *Molecular Plant Pathology* (Dean et al., 2012).

Many of these pathogens are able to infect different host tissues, while some are restricted to the roots and/or stem bases. For example *Phytophthora infestans* (potato late blight pathogen) is able to infect the tuber, leaf and stem tissue of potatoes (Fry, 2008). Another common soil-borne pathogen *Fusarium oxysporum* infects the roots of susceptible host plants and is able to cause wilt disease on the above ground parts of plant via infection and spread through the xylem tissue (Michielse & Rep, 2009). Other soil-borne pathogens are restricted to infection of specific tissue, such as the eyespot fungi *Oculimacula yallundae* and *O. acuformis*, which are confined to infection of the stem base (Lucas et al., 2000), and *Gaeumannomyces graminis* var. *tritici*, which infects root and stem base tissue (Skou, 1981).

Often yields decline when growing crop monocultures or short rotations. This has been associated with the build-up of soil-borne plant pathogens (Bennett et al., 2012). Take-all disease of wheat is the prime example of a soil-borne pathogen that builds-up during consecutive cereal cropping, causing significant yield loss.

1.2. Take-all disease of wheat

Globally, take-all is regarded as the most important root disease of wheat and when severe can be devastating to wheat productivity (Cook, 2003). Take-all was first described in Australia in the nineteenth century and has now been found to be widespread throughout the temperate wheat growing regions of the world as well as

occurring in some high altitude subtropical and tropical regions (Hornby et al., 1998). Take-all is caused by the soil borne fungus *Gaeumannomyces graminis* var. *tritici* (Ggt) (Walker, 1981). *Gaeumannomyces graminis* var. *tritici* also infects the cereals barley, triticale and rye (Asher & Shipton, 1981).

Typical take-all symptoms include black lesions on roots and stem base blackening on young wheat plants (Figure 1.1). In the field severe take-all can cause stunting and premature ripening of the crop (Figure 1.2). As a result grain filling will be poor and there can be significant yield losses (Schoeny et al., 2001). Grain from severely infected plants will also be shrivelled and of little use to millers (Manners & Myers, 1981). The grain quality parameters, thousand grain weight and Hagberg falling number, are both badly affected by severe take-all disease (Gutteridge et al., 2003).



Figure 1.1. Take-all lesions and stem base blackening on wheat seedlings.



Figure 1.2. Take-all patch showing stunting and premature ripening of the wheat crop.

In consecutive wheat crops take-all is typically absent from 1st wheats, most severe in years 2-4 and then declines. The latter phenomenon is referred to as take-all decline (TAD)(Slope & Cox, 1964). In the UK crops at risk from take-all disease (non-first wheat crops) can account for approximately 35 to 65% of the total wheat crop area in any one year (Hornby et al., 1998, Spink et al., 2002). Second wheats typically yield 1 to 1.5 tonnes/ha less than first wheat crops (HGCA recommended list, <http://www.hgca.com>). This 'second wheat syndrome' is thought to be primarily due to the effect of take-all disease. However, there are confounding factors; second wheat crops are probably sown later, trials will be situated on different sites, grass weed problems can be greater in continuous wheats, other diseases such as common root rot become more of a problem in consecutive wheat crops, and there could be effects of previous break crop or wheat crop on nutrient availability in the soil.

There is little data relating specifically to financial losses in the UK due to take-all. In England and Wales during the late 1980s losses were estimated at up to £55 million annually in second and subsequent wheat crops (Hornby et al., 1998). Yield and financial loss due to take-all in the UK are hard to determine due to difficulties in accurately assessing disease (Hornby & Bateman, 1990). Nationally, there is also no comprehensive long term monitoring of take-all disease making it hard to quantify the effects of take-all.

Take-all also has an important environmental consequence; severely infected plants leave unused nitrogen fertiliser in the soil which is available for leaching from the soil (Macdonald et al., 1997, Hornby et al., 1998, Macdonald & Gutteridge, 2012). Nitrate leaching can pollute drinking water sources, and has been linked to human health concerns (Ward et al., 2005b). There is EU legislation that sets a limit to the acceptable amount of nitrate in drinking water to 50 mg/l. Nitrates also cause serious environmental pollution (Addiscott, 2004). Nitrate leaching causes eutrophication of freshwater waterways and excessive algal growth in coastal and estuarine waters, killing fish and natural vegetation. The 1991 EU Nitrates Directive required that member states designate nitrate vulnerable zones (NVZs) for agricultural areas most at risk from nitrate pollution. Within these areas codes of good agricultural practice were established to minimise nitrate losses due to agriculture (http://ec.europa.eu/environment/water/water-nitrates/index_en.html). NVZs cover 62% of England, including all the major cereal growing regions of the country (Environment agency, <http://www.environment->

agency.gov.uk/). Effective control of take-all in these areas is important to protect the waterways from this additional source of excess nitrogen in the soil.

Take-all can largely be avoided by crop rotations away from susceptible cereal hosts. However, due to economic reasons, there has been a trend to increase the proportion of susceptible hosts in wheat-based rotations as the intensity of cropping increases (Cook, 2003), so take-all remains one of the most difficult and important pathogens to control.

1.3. UK wheat varieties

To be approved for sale in the UK wheat varieties must be included on the National List (NL). The National List system was introduced into the UK in 1973 and the Food and Environment Research Agency (Fera) is responsible for the National Lists of agricultural crops in the UK (www.fera.defra.gov.uk). To be accepted onto the national list wheat varieties must be trialled over a two year period and fulfil certain criteria to be **d**istinct, **u**niform and **s**table (DUS) and show **v**alue for **c**ultivation and **u**se (VCU).

Once wheat varieties are approved for sale and are marketed in the UK further independent field trials are carried out each year to compare the best varieties. Farmers can then receive information and advice from these trials to help choose which varieties to grow. Nabim (National Association of British and Irish Millers) classifies wheat varieties into one of four groups to provide an indication of the end use of the grain and potential price (www.nabim.org.uk). Group 1 varieties are bread wheat varieties with reliable milling and baking qualities. They attract a premium price above the base level. Group 2 varieties have bread-making potential, but are not as consistent as Group 1 and tend to have lower and more variable premiums. Group 3 varieties are generally soft varieties for use in biscuit and cake making. Group 4 varieties are the feed wheats. They are commonly high yielding but with poorer quality and little premium.

The HGCA Recommended List (RL) provides information to farmers on the performance of varieties in independent trials. Varieties on the RL 'are considered to provide a consistent economic benefit to the whole industry' (www.hgca.com). The varieties are evaluated based on yield in different situations; with or without fungicides, as a first or second cereal, early or late sowing date and light or heavy soil type. Disease resistance to powdery mildew (*Blumeria graminis*), yellow rust (*Puccinia striiformis* f. sp. *tritici*), brown rust (*Puccinia triticina*), glume blotch (*Septoria nodorum*), septoria leaf blotch (*Mycosphaerella graminicola*), eyespot (*Oculimacula* spp.), fusarium ear blight (*Fusarium* spp.) and orange wheat blossom midge (*Sitodiplosis mosellana*) are

also evaluated. Grain quality characteristics such as specific weight, and agronomic features such as crop height and earliness are also recorded. There is no information regarding susceptibility to take-all disease on the HGCA Recommended List.

1.4. The *Gaeumannomyces* genus

Gaeumannomyces graminis var. *tritici* is a homothallic ascomycete fungus within the family Magnaporthaceae and is related to another important fungal pathogen, *Magnaporthe oryzae* (the rice blast fungus). Within the *Gaeumannomyces* genus there are seven identified species: *Gaeumannomyces graminis*, *G. cylindrosporus*, *G. wongoonoo*, *G. caricis*, *G. incrustans*, *G. medullaris* and *G. amomi* (Freeman & Ward, 2004). *Gaeumannomyces graminis* and *G. cylindrosporus* have both been isolated from the roots of cereal and grass species (Hornby et al., 1977, Hornby et al., 1998). *Gaeumannomyces graminis* fungi can be highly pathogenic. In contrast *G. cylindrosporus* (anamorph *Phialophora graminicola*) are non-pathogenic fungi colonising the outer root cortex of cereals (Hornby et al., 1977, Walker, 1981). *Gaeumannomyces wongoonoo* causes patch disease of buffalo grass, *G. caricis* is pathogenic to sedges, *G. incrustans* infects turf grasses and *G. medullaris* has been isolated from the rush *Juncus roemerianus* in North America (black rush) (Walker, 1981, Landschoot & Jackson, 1989, Kohlmeyer et al., 1995, Wong, 2002). The seventh species, *G. amomi*, is a tropical endophytic fungus recovered from the healthy leaves and stems of wild ginger plants, *Amomum siamense* (Bussaban et al., 2001a, Bussaban et al., 2001b).

Within *G. graminis* there are four known varieties: var. *avenae*, var. *graminis*, var. *maydis* and var. *tritici*. All of the *G. graminis* varieties are pathogens of cereals or grasses but *Ggt*, the wheat take-all fungus, is the only pathogen of real global importance. *Ggt* primarily infects the cereals wheat, triticale, barley and rye. But a wide range of cereal relatives and grass species can also be infected by the fungus (Nilsson, 1969). *Gaeumannomyces graminis* var. *avenae* has a similar host range to *Ggt* but in addition is able to infect oats and also causes take-all patch of turf grass. *Gaeumannomyces graminis* var. *graminis* causes sheath blight of rice but is only very weakly pathogenic on other cereals while *Ggm* causes take-all disease of maize. Ascospore size and shape, hyphopodia (specialised hyphal branch structures formed to penetrate plant material) shape and host preferences are characteristics used to distinguish between *Gg* varieties (Hornby et al., 1998, Freeman & Ward, 2004).

Within populations of *Ggt* there are also two commonly identified major genetic sub-populations in the field (Daval et al., 2010). This is discussed in more detail in the introduction to Chapter 5. Different sub-populations of *Ggt* have been linked to pathogenicity and mycelial growth rate on wheat (Irzykowska & Bocianowski, 2008), disease severity (Wilocquet et al., 2008), and relative proportions of sub-populations correlated with different stages of take-all epidemics in cereal sequences (Lebreton et al., 2007).

1.5. Take-all infection process and epidemiology

In the absence of a living host *Ggt* survives saprotrophically on dead roots and stem bases and this forms the main source of inoculum for the next susceptible crop (Cook, 2003). *Gaeumannomyces graminis* var. *tritici* is a relatively poor saprotrophic competitor so that survival of inoculum rapidly declines when a break from a susceptible cereal in the rotation occurs. Inoculum will then build-up in the soil when a susceptible host crop is grown and can cause severe disease in a 2nd or subsequent susceptible cereal crop. *Gaeumannomyces graminis* var. *tritici* inoculum in the soil cannot spread far from its source (Wilocquet et al., 2008) but when disease is severe perithecia can be produced under conditions of high rainfall on the stem bases of plants or on stubble after harvesting (Walker, 1973, Hornby, 1981). The perithecia produce ascospores which, when released and carried by the wind, can contribute to the initial inoculum at the beginning of an epidemic and potentially spread disease further afield. However, ascospores are generally considered only minimally important as a source of inoculum in take-all epidemics (Hornby, 1981, Freeman & Ward, 2004).

Primary infection of winter wheat occurs as inoculum that has survived on crop residues in the soil comes into contact with the roots of newly germinating seeds (Brown & Hornby, 1971, Hornby, 1981, Bailey & Gilligan, 1999). Hyphae are able to grow trophically over short distances towards roots if direct contact does not occur (Skou, 1981). From the initial infection site runner hyphae grow and spread along the root surface in all directions. Runner hyphae are thin and transparent but as they age turn a darker brown colour. Hyaline branches from the runner hyphae form infection hyphae and these penetrate root epidermal cells. Penetration pegs (hyphopodia) form at the tips of infection hyphae and, as the result of mechanical pressure, allow penetration of the cell wall and invasion of the epidermal cells (Skou, 1981). There is also evidence that toxins are produced when *Ggt* hyphae penetrate into root cells (Skou, 1975a). The infection hyphae then spread quickly throughout all parts of the root system and destroy

the vascular tissue. Invasion of the vascular tissue has been shown to reduce water and nutrient uptake from the soil (Asher, 1972, Pillinger et al., 2005), which leads to the typical above ground symptoms and associated yield losses. Black lesions can be seen on infected roots where the vascular tissue has been destroyed (Figure 1.3). Runner hyphae can also spread onto the seed and lower stem base.



Figure 1.3. Dark runner hyphae of the take-all fungus on the surface of a wheat root and black take-all lesions within the root tissue. Magnification: x65.

Primary infections decrease as inoculum decays and secondary infections due to root to root contact then become more important (Gilligan et al., 1994, Bailey et al., 2005). In winter wheat crops, sown in the autumn, secondary infections typically occur from the beginning of spring and continue throughout the rest of the growing season (Hornby et al., 1998). Secondary infections increase considerably in late spring and into summer probably due to the increase in rooting density as the season progresses so that more root to root contact occurs (Brassett & Gilligan, 1989). After the crop has been harvested the infected crop residues in the soil remain and can continue the cycle of infection if a new susceptible crop is planted.

Take-all occurs worldwide and can develop over a wide range of physical conditions; it is reported to develop in soils from pH 5.5 to 8.5 and where soil temperatures range from 5°C to 30°C (Hornby, 1981, Hornby et al., 1998). Take-all survives in a range of soils but generally well-structured soils with better aeration favour take-all development. Soil moisture also favours this disease (see below).

Take-all is a notoriously patchy and unpredictable disease in the field (Hornby, 1978, Clarkson & Polley, 1981, Cotterill & Sivasithamparam, 1989b, Roget & Rovira, 1991, Bateman & Hornby, 1995, Hornby et al., 1998, Oliver et al., 2003, Gosme et al., 2007). Bateman and Hornby (1999) suggested that the unpredictability is due to the influence of the weather, soil conditions, agronomic management practices such as soil cultivations, cropping history and host-disease-inoculum interactions. Hornby et al. (1998) state that severe take-all years are generally associated with seasonal weather patterns and the environment can have a strong influence on disease. High rainfall in the spring and summer has been linked to more severe outbreaks of take-all (Clarkson & Polley, 1981) and a generally high soil water potential is thought to be essential for *Ggt* fungal growth (Cook, 1981). Pillinger et al. (2005) also found high soil moisture levels to be associated with more severe take-all epidemics. In Australia, Roget and Rovira (1991) found the development of take-all and associated yield losses to be strongly correlated with spring rainfall and that rainfall in the previous season influenced take-all in the next season. Hornby (1978) investigated relationships between the weather and take-all based on 33 years of descriptive information on take-all disease at Rothamsted Research, UK. Take-all disease was split into four categories: rare, prevalent, damaging or severe. Damaging take-all years were associated with warm, dull springs and warm but dry summers while severe take-all years were associated with lower summer temperatures and high rainfall. Hornby et al. (1998) report that in 1987 dry weather in the early summer put plants under additional stress, resulting in higher than expected disease symptoms above ground (damaging take-all), compared with take-all infection of the roots. Based on this information it is generally accepted that wet weather encourages take-all development while dry weather in the summer can exacerbate the effects of take-all on the crop (Hornby et al. 1998).

Temperature has also been linked to the development of take-all (Hornby, 1978). In the autumn, high temperatures and humidity have been associated with more severe disease due to increased root growth and mycelial growth which enhance the probability of primary infections occurring (Ennaifar et al., 2007). Conversely, delay in the development of disease in the field has been linked to cold weather (Bailey & Gilligan, 1999) which restricts mycelial growth.

Environmental factors such as temperature and soil moisture also affect *Ggt* inoculum surviving saprotrophically in the absence of a host crop (Macnish, 1973, Shipton, 1981, Wong, 1984, Cotterill & Sivasithamparam, 1987a, Bithell et al., 2009). This is

important as the initial amount of inoculum surviving in the soil when a susceptible crop is sown influences the risk of severe take-all disease developing (Hornby, 1981). *Gaeumannomyces graminis* var. *tritici* is a poor saprotrophic competitor and dry, cold weather, which inhibits microbial activity, is known to slow down the breakdown of inoculum. While warm, moist soil generally increases the rate of inoculum decline.

Many soil borne organisms show variation in their spatial distribution in the field (Ettema & Wardle, 2002) and soil-borne plant pathogens often cause patchy diseases (Belmar et al., 1987, Hornby et al., 1998, Truscott & Gilligan, 2001, Stacey et al., 2004). Take-all disease patchiness is likely to be due to environmental conditions and/or crop management practices such as soil cultivations. Local soil conditions within a field such as soil type, aeration and drainage may exacerbate patchy above ground symptoms of take-all (Catt et al., 1986, Hornby et al., 1998). Some grass weeds including annual bromes have been shown to maintain take-all inoculum in the soil (Gutteridge et al., 2006). If grass weeds are not effectively controlled in a break crop then the position of these weeds in a field could lead to development of take-all patches. White (1945) reported that the size, shape and position of take-all patches changed in the first two successive wheat cropping seasons after a break. In subsequent wheat crops the take-all patches were less defined and take-all covered the majority of the area. R.J. Gutteridge (personal communication, 2009) also found that in sequential cereal crops the take-all patches do not develop in the same location in individual fields. White (1945) suggests that local soil conditions could influence the size and position of patches. However, he found no difference in soil pH, organic carbon, nitrogen or soil texture between healthy and take-all patch areas. White (1945) suggested that other factors such as availability of trace elements could be influencing disease. The more uniform disease as consecutive wheat cropping continues is suggested to be due to the spread of *Ggt* inoculum throughout the field from initial foci in the first wheat crop. Truscott and Gilligan (2001) propose that mechanical soil cultivations are important for the dispersal of soil-borne pathogens. These soil cultivations can also affect the location of disease patches from year to year as inoculum in patch areas is diluted and spread to other areas.

1.6. Take-all control methods

1.6.1. Cultural control

Cultural methods of control of take-all disease have been extensively investigated, in part probably due to a lack of effective and reliable chemical control or genetic

resistance (Cook, 2003). A number of agronomic practices can influence disease. Crop rotation is the oldest and most effective form of cultural control. If a break crop away from a susceptible cereal host is taken then the risk of disease in the following wheat crop is usually negligible. This is due to the poor saprotrophic survival of *Ggt* in the absence of a host crop. Non host crops such as oilseed rape, linseed, peas, and beans are effective break crops and practically eliminate take-all in the following wheat crop (Yarham, 1981). However, crop rotation as a control measure relies upon the destruction of wheat volunteers that if left can carry take-all inoculum through a break crop putting a following first wheat crop at risk of disease (Jenkyn et al., 1998).

Grass weed infestations in break crops can also reduce the effectiveness of the break to control take-all. Many grass species, both cultivated and wild, are reported as hosts of *Ggt* (Nilsson, 1969). Dulout et al. (1997) reported that the occurrence of blackgrass (*Alopecurus myosuroides*) in set-aside fields increased the risk of severe take-all in the following wheat crop. Annual brome grasses (*Bromus* spp.) have also been reported as maintaining take-all inoculum in the soil during a break (Gutteridge et al., 2006).

Gutteridge and Hornby (2003) reported that delayed sowing of winter wheat from mid-September to mid-October under UK conditions reduced take-all severity in a second wheat crop, provided that wheat volunteers were controlled during this time. An earlier study by Prew et al. (1986) also found that third wheat crops sown in mid-October had less take-all. This is in part due to the decline of *Ggt* inoculum in the absence of a host crop in the longer inter-crop period. Lower disease levels for late sown crops may also be due to the shorter time period for autumn infection (Colbach et al., 1997). Prew et al. (1986) found that third wheat crops sown in mid-October had less than 20% plants infected in December, compared with over 80% of plants infected in crops sown in mid-September. However, the disadvantage of delaying sowing is that the yield potential of the crop decreases because of the shorter growing season (Cook, 2003).

Cultivation practices are also reported as influencing take-all incidence in a second wheat crop (Cook, 2003). There has been a trend towards minimum tillage/direct seeding in recent years but the influence of this on take-all is unclear. Direct seeding has been reported as increasing take-all incidence in some instances (Kordas, 2006) but a decrease in take-all has also been reported (Rothrock, 1987). Another study reported no difference in take-all incidence between conventional tillage and direct seeding (Schroeder & Paulitz, 2006).

Crop nutrition has been implicated as important in minimising take-all disease. Phosphate deficient soils are generally considered to encourage more severe disease (Hornby et al., 1998). However application of phosphorus to soil where inoculum is already present does not seem to prevent severe disease in the following crop (Gutteridge et al., 1996), suggesting that any phosphate deficiency in the soil should be corrected before sowing a susceptible crop. There is less information on potassium, manganese and sulphur deficient soils, but these deficiencies are also regarded as favouring take-all although severe disease can still develop in soils that have a good supply of these nutrients (Hornby et al., 1998).

The type and timing of nitrogen fertilisation can influence disease. Ammonium fertilisers have been shown to reduce disease compared with nitrate fertilisers (Sarniguet et al., 1992) and a high ammonium content was better than a low ammonium content in another study (Colbach et al., 1997). Lucas et al. (1997) found that applications of ammonium fertilisers were most effective at reducing disease when applied earlier in the growing season, perhaps to promote early root growth. Nitrogen fertilisers containing chloride are reported as helping to minimise yield loss in take-all situations but did not directly influence the severity or occurrence of disease (Christensen et al., 1990). Chloride containing fertilisers may therefore help improve the tolerance of host plants to infection. The effectiveness of chloride containing fertilisers is not clear as another study indicated no real benefit of chloride containing fertilisers (Werker & Gilligan, 1990).

1.6.2. Biological control

Take-all decline (TAD)

In consecutive wheat crops take-all disease usually peaks in years 2-4 and then in any subsequent crops disease severity is reduced as the soil becomes naturally suppressive to take-all. This phenomenon is called Take-all Decline (TAD) (Slope & Cox, 1964, Slope & Etheridge, 1971, Shipton et al., 1973, Cook, 2007). This decline in take-all is a natural form of specific disease suppression that develops in wheat and barley monoculture. It has often been reported in fields in the UK and Europe, as well as America, but has rarely been reported in Australia. The hot and dry environmental conditions in Australia have been suggested as being unfavourable for the development of take-all decline (Yarham, 1981).

In field experiments TAD has been established in fields with both artificial and natural inoculum sources (Bateman & Hornby, 1999). It is generally agreed that the natural development of take-all suppressive soils is due to a shift in the antagonistic microbial community (Weller et al., 2002). Different microbes and suppressive mechanisms may be responsible for the development of TAD in soils from different regions (de Souza et al., 2003). Several fluorescent *Pseudomonad* spp. have been isolated from TAD soils and production of the antimicrobial compound 2,4-diacetylphloroglucinol (2,4-DAPG) by these species has been commonly implicated in suppression of *Ggt* (Mazzola et al., 1992, Weller et al., 2007). In another study Actinomycetes, *Pseudomonas* spp. and several different fungi have been implicated in suppression of disease in TAD soils in Montana (Andrade et al., 1994). Both mycoparasitism and antibiosis have been identified as mechanisms of suppression operating in these soils. More recently Sanguin et al. (2009) demonstrated that the composition of the rhizobacterial community changes with the different stages of take-all decline and they suggested that TAD may be due to complex interactions and changes in the total bacterial community composition, rather than just due to the antagonistic *Pseudomonas* species.

Environmental factors such as weather, soil type and geographical location affect the development of TAD. When other conditions are favourable to infection and disease development, take-all can still cause significant losses in wheat crops even when TAD is established (Hornby et al., 1998).

While TAD is an example of an induced specific form of suppression that develops during continuous wheat cropping there are other soils that are naturally suppressive to *Ggt* but are not associated with wheat monocultures (Cook & Rovira, 1976). Suppressives soils are also common for other soil-borne diseases, such as Fusarium wilt (Rouxel et al., 1979, Mazurier et al., 2009), Rhizoctonia root rot (Henis et al., 1979) and Pythium root rot (Knudsen et al., 2002). Natural suppressiveness of soils to disease has been explained by physical and chemical properties of the soil and/or as a result of the action of soil microbes (Hoper & Alabouvette, 1996, Adioboa et al., 2007).

***Phialophora* spp.**

Phialophora fungal species belonging to the *Gaeumannomyces-Phialophora* (GP) complex (Freeman & Ward, 2004) have been implicated in induced and general suppression of take-all disease. The *Phialophora* species in the GP complex are all anamorphs of *Gaeumannomyces* species, and they can grow in or on the roots of cereal

and grass species. In the UK a delay in the development of take-all epidemics has been demonstrated due to the presence of *Phialophora graminicola* (teleomorph *Gaeumannomyces cylindrosporus*), populations of which expanded in grass leys over two or more years prior to wheat being sown (Deacon, 1973a, Slope et al., 1979). *Phialophora graminicola* has also shown control of *Ggt* in pot experiments (Gutteridge & Slope, 1978). *Phialophora graminicola* itself is only quite weakly pathogenic and does not cause any significant damage to cereal plants under field conditions (Hornby et al. 1998). The mechanism by which *P. graminicola* reduces take-all disease is not known. Hornby (1983) suggested that it is a host response to infection that could be responsible because there is no evidence of an effect of *P. graminicola* to *Ggt* on agar plates and prior colonisation of wheat roots by *P. graminicola* is required for effective control. Competition between these fungi for food resources in root tissues has also been proposed as a mechanism of cross-protection (Hornby et al., 1998). Only very low levels of *P. graminicola* have been detected in TAD soils in the UK, suggesting that the delay in the onset of take-all epidemics and the decline of take-all in wheat monocultures are affected by different biological control processes (Slope et al., 1978).

Phialophora sp. lobed hyphopodia (teleomorph *G. graminis* var. *graminis*) has also been shown to control *Ggt* in pot experiments (Deacon, 1974). Under field conditions Martyniuk & Myskow (1984) reported on partial control of *Ggt* by artificially introduced *Phialophora* sp. lobed hyphopodia against low to moderate levels of *Ggt*. Unlike *P. graminicola*, populations of *Phialophora* sp. lobed hyphopodia are not reported to develop under grass leys. The fungus does occur naturally on wheat roots under field conditions but the factors that encourage these populations to develop are not known (Gutteridge et al. 2006).

Wong (1975) and Wong and Southwell (1980) demonstrated that isolates of *Ggg* were effective at reducing take-all in glasshouse and field experiments when they were used to pre-colonise wheat roots. More recently Gutteridge et al. (2007) investigated the potential of *G. cylindrosporus* and *G. graminis* var. *graminis* as take-all biocontrol agents under field conditions. Natural populations of *G. cylindrosporus* were more effective at controlling take-all, but artificially introduced inoculum also sometimes reduced take-all. *Gaeumannomyces graminis* var. *graminis* was not a very effective biocontrol agent in this study.

Wong et al. (1996) showed good field control and yield increases of 21-45% when isolates of *Ggg* or *Phialophora* sp. lobed hyphopodia were applied before sowing.

Wong et al. (1996) stated that two of the isolates tested had been patented for biocontrol and were going to be developed for commercial use. However, their commercial use has never subsequently been documented.

Introduced biological control agents

A number of different microorganisms have been isolated from the soil or wheat roots and examined as potential biological control agents for *Ggt* (Hornby et al., 1998). The large amount of literature on this subject has been previously reviewed (Wong, 1981, Hornby et al., 1998, Cook, 2003). Examples of some of the most common biocontrol agents investigated are described below.

Often potential biocontrol agents have been isolated from soils known to be suppressive to take-all (including TAD soils). Commonly isolated bacteria from these soils include *Bacillus* spp. and fluorescent *Pseudomonas* species. *Pseudomonas fluorescens* strains have been reported as suppressing take-all in pot and field experiments (Duffy & Weller, 1995, Chapon et al., 2002, Sari et al., 2008). *Pseudomonas fluorescens* strains have been shown to control take-all by colonising the root surface and producing a range of antibiotics such as 2,4-DAPG. Okubara and Bonsall (2008) have demonstrated that the accumulation of 2,4-DAPG on the surface of wheat roots depends on the wheat cultivar and also that there are host genotype-bacterial strain interactions. A non-fluorescent *Pseudomonas* strain AN5 (*Ps.* AN5) has also been reported as an effective biocontrol agent (Kaur et al., 2006). The fungal metabolite D-gluconic acid, produced by *Ps.* AN5, was identified in this study as the predominant metabolite responsible for the control of *Ggt*. Most recently Daval et al. (2011) demonstrated that infection of wheat roots with *Pseudomonas fluorescens* Pf29Arp down-regulated several *Ggt* fungal genes and up-regulated other plant genes suggesting that this *Pseudomonas* strain inhibits *Ggt* by disrupting fungal infection and through induction of host plant defences.

In China *Bacillus* spp. have been sold commercially since the 1980s for the promotion of crop growth. In Australia two species (*Bacillus subtilis* and *B. cereus*), originating from rhizosphere soil in China, have shown potential for biocontrol of take-all in Australian soils under glasshouse conditions (Ryder et al., 1999). In these experiments some strains of *Bacillus* reduced take-all severity by up to 40% after four weeks. More recently, Liu et al. (2009) demonstrated that a *B. subtilis* strain (E1R-j) was an effective biocontrol agent under glasshouse and field conditions when applied as a soil drench, reducing take-all severity by over 55% in field experiments. Another species, *Bacillus*

pumilus (strain 7km), isolated from soil in Iran is reported as reducing the severity of take-all disease by inducing host resistance (Sari et al., 2007). Screening for biocontrol agents in pot experiments is useful but is not always reliable in predicting potential use in the field; usually under field conditions results are more variable. In the early 1990s a series of field trials in the UK with one *B. cereus* var. *mycooides* and a *B. pumilus* strain applied as soil drenches were ineffective at controlling take-all (Hornby et al., 1993).

Fungi identified as potential biocontrol agents are arbuscular mycorrhizal fungi (AMF). Both Graham and Menge (1982) and Khaosaad et al. (2007) have shown in controlled environment conditions that take-all disease is reduced due to AMF root colonisation. Castellanos-Morales et al. (2011) recently demonstrated that the amount of AM root colonisation by *Glomus mosseae* and the level of protection provided also depended on the variety of barley tested.

Other fungi identified as potential biocontrol agents belong to the genus *Trichoderma*. *Trichoderma* are ascomycete soil dwelling fungi that have often been used as biocontrol agents of plant pathogens (Verma et al., 2007). Recently, in Iran, a selection of *Trichoderma* isolates and two commercial *Trichoderma* bioproducts have been screened as potential biocontrol agents of take-all (Zafari et al., 2008). In the greenhouse some isolates reduced disease severity by up to 55%. *Trichoderma* isolates have been shown to suppress take-all in a number of ways including competition for space, antibiosis and hyperparasitism. However, in the field biocontrol is a much bigger challenge because of the heterogeneous nature of the soil environment and interactions with other microorganisms. So far the biocontrol of take-all using introduced bacterial or fungal biological control agents is often inconsistent in the field and so not economically viable (Weller et al., 1988, Cook, 2003).

1.6.3. Chemical control

Two fungicides fluquinconazole and silthiofam are currently commercially available as seed treatments and show good activity against take-all. Fluquinconazole is a quinazoline-based triazole fungicide which inhibits sterol biosynthesis in fungal cell membranes (Dawson & Bateman, 2000). Seed treatment with fluquinconazole (commercially available as Jockey® since 2000) can effectively control moderate take-all and increase grain yields. However, when take-all is more severe there is only a relatively small yield improvement and grain quality is still poor (Bateman et al., 2004). Fluquinconazole applied as a seed treatment has the additional benefit that it also

provides effective control of seed-borne plant pathogens including *Ustilago* spp. and *Tilletia* spp. (Wenz et al., 1998).

Silthiofam, applied commercially since 2001 in the formulation Latitude®, has consistently been shown to reduce disease when applied as a seed treatment in field trials (Schoeny & Lucas, 1999, Spink et al., 2002), and has been shown to be more effective than fluquinconazole at improving yields (Bateman et al., 2008). Epidemiological modelling has shown that silthiofam significantly reduces primary infection but has little effect on secondary infection (Bailey et al., 2005). This indicates that control of disease occurs early in the disease cycle restricting the epidemic rather than because of long term action of silthiofam during the growing season (Bailey et al., 2005). Silthiofam is specific to *Ggt* and probably inhibits ATP transport from mitochondria (Joseph-Horne et al., 2000). Research shows that application of this fungicide causes degeneration of the cytoplasm in hyphal cells leading to cell death and can also enhance the defence reaction of the host to fungal invasion of the roots (Huang et al., 2001). Different 'naïve' *Ggt* isolates from the field show large differences (10,000 fold) in the concentration of silthiofam required to inhibit growth (Joseph-Horne et al., 2000) and some isolates are naturally resistant/insensitive (Carter et al., 2003).

Two strobilurin fungicides, which act by disrupting ATP formation and so inhibiting mitochondrial respiration, have also shown some potential for the control of take-all. Foliar sprays of azoxystrobin have been shown to decrease take-all severity and increase yields (Jenkyn et al., 2000), but results can vary considerably in field experiments and so azoxystrobin has previously been considered too inconsistent for control (Bateman et al., 2006). Another strobilurin, fluoxastrobin, in its commercial formulation Fandango® is currently recommended for providing additional control of take-all when applied as a spring drench.

1.7. Genetic control

The use of resistant germplasm as a means to control disease is an attractive option because it can reduce the use of pesticides and also requires no further costs to the farmer. It also strengthens food security in areas of the world where crop losses can be high and chemical control is not readily available and/or is too expensive. Sources of resistance that have been previously exploited in other crop-pathogen systems include current commercial varieties, older landraces and ancestral wild relatives of the crop species. Despite a range of cultural, biological and chemical control options for take-all

the disease remains a problem. The identification of resistant germplasm could help provide more durable disease control, significantly reduce yield losses due to take-all 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). Other related species display differences in their susceptibility to take-all although none so far have been successfully utilised to improve the resistance of wheat.

1.7.1. Resistance of oats to take-all

Oats (*Avena*) are a non-host to *Ggt*; this feature is attributed to production of the antifungal compound avenacin in plant tissues. Avenacin is a plant secondary metabolite that provides broad-spectrum defence to soil-borne pathogens (Papadopoulou et al., 1999). Osbourn *et al.* (1994) demonstrated that a diploid oat species (*Avena longiglumis*) that lacked detectable avenacin was susceptible to *Ggt*. Oats are however susceptible to *Gaeumannomyces graminis* var. *avenae*. This variety of *Gaeumannomyces* has been identified as producing the enzyme avenacinase, which can convert avenacin to a less toxic form and so allow infection (Osbourn et al., 1991).

Avenacin production is absent from wheat, barley and rye (Osbourn, 2003). Qi *et al.* (2004) reported on the presence of a gene cluster in the oat genome encoding three or more different biochemical steps in the synthesis of avenacin. The gene cluster has now been defined and mutants characterised to study functional activity in the diploid oat species *Avena strigosa* (Mylona et al., 2008, Mugford et al., 2009, Wegel et al., 2009). However, biosynthesis of avenacin is complex and the pathways and genes controlling production are not fully characterised. Oat is also not closely related to wheat, making introgression of the avenacin gene cluster particularly difficult. However, the transgenic option is being considered (A. E. Osbourn, JIC, personal communication). Most recently Inagaki et al. (2011) have expressed the gene in rice for the first step in the avenacin pathway, resulting in the production of the simple triterpene β -amyrin.

If wheat could be genetically engineered to produce avenacin this would provide a source of complete immunity to *Ggt*. However, the durability of this source of resistance has been questioned. It has been proposed that this would lead to selection of *Ggt* isolates that were insensitive to avenacin and/or increase the occurrence of *Gga* isolates infecting wheat plants (Cook, 2003). Good field performance of genetically modified wheat producing avenacin therefore seems fairly uncertain in the long term.

1.7.2. Resistance of rye to take-all

Rye (*Secale*) is reported to be far more resistant to *Ggt* infection than wheat, and the wheat x rye hybrid triticale generally shows an intermediate susceptibility between wheat and rye (Nilsson, 1969, Jensen & Jorgensen, 1973, Scott, 1981, Hollins et al., 1986, Rothrock, 1988, Solel et al., 1990, Gutteridge et al., 1993, Gutteridge et al., 2003, Bithell et al., 2011a). The resistance of rye to take-all is considered partially a result of the greater capacity of rye to produce new roots than wheat and barley, thus allowing some degree of disease escape (Skou, 1975b). Tissue based resistance is also implicated by observations that the total extent of root discolouration is lower in rye (Skou, 1975b). Field experiments show that the disease epidemic on the roots progresses slower in rye than wheat and barley (Gutteridge et al., 1993). The intermediate resistance of triticale to *Ggt* has been demonstrated in a variety of different triticale cultivars and also at both high and low disease pressures (Hollins et al., 1986). Hexaploid triticale varieties (genome AABBRR) were a little more resistant than octoploid varieties (genome AABBDDRR). Researchers suggest that the greater susceptibility of octoploid triticale varieties is because a larger proportion of the genome is from wheat compared with the contribution from rye (Scott et al., 1989). Long term field experiments at Rothamsted Research have shown that switching to triticale instead of wheat can reduce the severity of take-all found compared with that expected if another wheat crop is grown (Hornby & Gutteridge, 1995). However triticale does not act as an effective break and when wheat growing is resumed severe disease still develops.

The mechanism of the tissue resistance in rye is so far unclear. Wilkes et al. (1999) have suggested that the production of hydroxamic acids by different cereal species is involved in susceptibility to take-all. Hydroxamic acids in a variety of plant species have been linked to insect and pathogen resistance. While the major hydroxamic acid in wheat roots is DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), the hydroxamic acid DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one) is also found in rye. *In vitro* studies of root extracts from wheat and rye show that rye root extracts inhibited *Ggt* growth more than wheat. When DIMBOA or DIBOA were directly incorporated into the growth media for *Ggt* DIBOA was more effective at inhibiting *Ggt* growth than DIMBOA (Wilkes et al., 1999). Wilkes *et al.* (1999) therefore attributed the greater resistance of rye to the presence of both DIMBOA and DIBOA, as opposed to just DIMBOA in wheat. However, the concentrations of DIMBOA and DIBOA used in

these *in vitro* experiments are outside the physiological ranges so far identified in cereal roots.

The genetic basis of resistance is also unknown. Although genetic exchange between rye and wheat is relatively difficult it is possible by conventional breeding to transfer whole rye chromosomes into wheat. For example chromosome substitutions have led to a wide range of disease resistance genes against powdery mildew and leaf rusts being transferred into wheat from rye (Schlegel & Korzun, 1997). In the case of take-all introduction of single rye chromosomes into wheat chromosome addition lines was unsuccessful at transferring resistance from rye to wheat, suggesting that resistance in rye is polygenic and involves multiple chromosomes (Hollins et al., 1986). There has not been any reported variation between rye varieties in their resistance to take-all so it has not been possible to investigate the genetic basis of resistance in this species.

1.7.3. Resistance of other grass species

Several grass species have been identified as partially resistant to take-all but the genetic basis has not been elucidated. Linde-Laursen *et al.* (1973) reported that both goat grasses (*Aegilops* spp.) and a diploid grass from another family *Hayaldia* (*Dasypyrum*) *villosum* show resistance to take-all. Both *Aegilops* and *Hayaldia* spp. are relatively closely related to wheat so that fertile hybrids can be formed between some genotypes (Scott, 1981). This would provide a route of introgression if suitably resistant material was identified.

Aegilops squarrosa (syn *A. tauschii* or *Triticum tauschii*) is a diploid wheat ancestor of common wheat that has the D genome. In the late 1980s *A. squarrosa* chromosome substitution lines generated using the highly susceptible wheat variety Winalta were tested for resistance (Conner et al., 1988). Results showed that the 6D single chromosome substitution improved resistance but the resistance was only effective at low to medium inoculum concentrations. Following on from this the Winalta-*A. squarrosa* 6D substitution line was crossed with several spring wheat varieties but the research was eventually discontinued (R.L. Conner, 2009, personal communication). Eastwood et al. (1993) also evaluated the susceptibility of 398 lines of *T. tauschii* to take-all. A small number of the lines showed less tissue blackening than the susceptible hexaploid wheat used in the study (cv Condor). However when these *T. tauschii* accessions were crossed with a tetraploid wheat parent (*Triticum turgidum* var. *durum*) the reduction in tissue blackening was no longer observed.

In Israel resistance to *Ggt* has been investigated in the wild emmer wheat (*Triticum dicoccoides*). In a laboratory assay the majority of wild emmer wheat accessions were moderately or highly susceptible but two accessions were identified as moderately resistant (Solel & Anikster, 1988). There seems to have been no published follow up study to this so it is probable that this source of resistance was not considered useful for control.

Hordeum grass species have also been evaluated for resistance. The *Hordeum* genus is comprised of annual and perennial grasses including barley. Barley itself is generally considered to be slightly less susceptible than wheat to take-all, similar to the susceptibility of triticale (Scott, 1981, Gutteridge et al., 1993, Hornby et al., 1998). In Denmark, up to 266 accessions of *Hordeum* spp. were evaluated but little difference in susceptibilities were found within and between species (Jorgensen & Jensen, 1976).

In another grass genus, *Agropyron*, two species were identified in the 1970s as exhibiting resistance similar to that of rye (Halloran, 1974, Scott, 1981). However, this not does seem to have been investigated further and hybridisation with hexaploid wheat is not easily achievable, thereby limiting the use of such material in breeding programmes. Overall, it is difficult to incorporate a single character such as take-all resistance from a wild grass species in a commercial cultivated cereal species, because of numerous other differences.

1.7.4. Resistance of wheat (*Triticum aestivum*) to take-all

Varietal differences in the susceptibility of hexaploid wheat to take-all have been previously reviewed by Scott (1981). For example in the 1970s glasshouse screening of over 1200 wheat varieties with different *Ggt* isolates identified only 30 varieties that were less susceptible than the current commercial Swedish varieties of the time (Mattsson, 1973). Differences were however only small and the varieties were not considered sufficiently better to be of use in breeding programmes. In Germany screening of over 2000 wheat species and varieties found that all of the lines were highly susceptible except from several of the *Triticum monococcum* lines which, again, were only slightly less susceptible (Mielke, 1974). Mielke (1974) also screened other rye, triticale and grass species for susceptibility and reported that some small differences were found. Often comparison of greenhouse and field trials with the same varieties did not correspond. The very detailed study by Nilsson (1969) is one of the only studies which demonstrates more consistent differences between wheat varieties

(Scott, 1981). Nilsson (1969) studied the susceptibility of over 100 wheat and barley varieties (24 varieties of winter wheat, 35 varieties of spring wheat, 55 varieties of barley and two wheat x rye hybrids) under natural take-all disease pressure in 10 replicated field trials. The degree of infection was measured by grading the severity of take-all on plant samples. Some wheat varieties were consistently less susceptible and had similar disease levels to triticale. Disease severity was also correlated with grain yield. Nilsson (1969) often found that the most resistant varieties had a larger number of crown roots and proposed that differences were primarily due to root system size. Since this study Scott (1981) reports that some of the least susceptible material has been tested by other researchers but often the results did not confirm their relative resistance. Nilsson's (1969) results do show that there are real differences between varieties, but it is likely that there is a very strong interaction with the environment and it is not clear that these differences are big enough to be useful in wheat breeding programmes. For any resistance to be useful it needs to be reliably expressed over sites and seasons under field conditions.

Since Scott (1981) reviewed the literature on host susceptibility to take-all there have been several other studies in the following decades. Field experiments in Germany found that the hexaploid wheat variety, cv Fakta, usually developed the least take-all infection over 6 years of collected data (Wachter, 1984). However, consistency between years was variable, again indicating a strong environmental interaction. Studies of resistance to *Ggt* have also been made in Australia. Penrose (1985) demonstrated that a single *Ggt* isolate penetrated the vascular root tissue of wheat varieties at different rates. Wheat seedlings were grown in sand for 10 days and the penetration of root tissue assessed by taking transverse sections of root tissue, staining the tissue and scoring for hyphal colonisation in different cell layers. Again, differences between varieties in these seedling tests were relatively small and not always significant. However, under natural field conditions the percentage of roots infected with take-all was found to differ between wheat varieties at two separate field sites (Penrose, 1991). Four years later a more detailed investigation reported on differences that were also independent of site between two hexaploid wheat varieties, Temu89-72 and cv. Bayonet, grown in five field trials over three years (Penrose, 1995). *Gaeumannomyces graminis* var. *tritici* infection was measured as the percentage of diseased seminal and crown roots at tillering and anthesis. Temu89-72 had around double the percentage of crown roots infected at anthesis than Bayonet. However, there was no data reported on the effect of this difference on yield or above ground symptoms of take-all for the two varieties. In

contrast to previous studies this research demonstrated quite large varietal differences that were consistent over sites. A mapping population between Temu89-72 and Bayonet was then made to do fieldwork to investigate the genetic basis of resistance, but funding proposals to actually do the work were unsuccessful (L.D.J. Penrose, 2009, personal communication).

Eastwood et al. (1994) used a different approach to finding novel sources of resistance in wheat by creating somaclonal variants from callus culture and then testing for resistance. Using this method variants were identified that had lower levels of tissue blackening in response to take-all infection. But this was not stably inherited between generations.

Comparison of above-ground symptoms of take-all and yields of wheat varieties suggests that varieties may also differ in their tolerance to take-all disease. In the USA there is evidence of partial tolerance to take-all of older hard red winter wheat varieties compared with the newer soft white winter wheat varieties (Huber & McCaybuis, 1993). The extent of take-all disease symptoms was similar between the two groups but yields of the hard red varieties were reduced less than the soft wheat varieties. Huber and McCaybuis (1993) suggest that this reflects the greater nutrient content of hard red wheat seeds so that these plants have a lower nutrient requirement from the environment and so are less affected by take-all.

In Australia resistance and tolerance of wheat varieties to take-all has been linked to the ability of wheat varieties to utilise the nutrient manganese. Rengel et al. (1993) provided evidence for a relationship between the manganese (Mn) efficiency of wheat varieties and resistance suggesting that Mn efficient plants were more resistant to take-all. The explanation of this relationship was attributed to the Mn efficient plants having in turn a more efficient process of conversion of phenolic compounds to lignin (Rengel et al., 1994). Lignin has been identified as having a key role in plant defence against plant pathogens. However neither levels of phenolics or lignin were significantly linked to infection with *Ggt*. Other Australian researchers have also linked take-all resistance in wheat to manganese availability (Graham & Rovira, 1984, Wilhelm et al., 1987). In the USA wheat grown from seeds containing high levels of Mn had less severe disease than plants from the same varieties with lower manganese seed content (Roseman & Huber, 1990). There is no evidence to suggest this is an important factor contributing to disease resistance in the UK.

Disease induced root growth has previously been implicated in the differing tolerance of wheat varieties to take-all (Scott, 1981). The production of extra roots when attacked by *Ggt* or a high intrinsic rate of root production is suggested as partially offsetting the loss of root function in already infected roots so that the plant can better tolerate infection. However, high root numbers could also increase the level of take-all infection as the probability of contact with the fungus in the soil increases at the beginning of the season (Colbach et al., 1997). Take-all is only able to extend short distances in the soil by mycelial growth so that as rooting density increases take-all could spread more quickly by secondary infections. So, while a low root density may help reduce primary infection or limit secondary spread, a high root density could help the host tolerate infection. Rooting pattern in the soil therefore has a complex effect on take-all development and host tolerance. Significant differences in disease-induced root production have been reported between two wheat varieties, Savannah and Genghis (Bailey et al., 2006). This study used highly controlled environmental conditions and epidemiological modelling to identify different host root production responses to *Ggt*. Under these conditions the variety Genghis showed an increase in disease induced root growth and an associated increase in the level of secondary root infections. The researchers suggest that environmental heterogeneity in the field would make such differences unlikely to be detectable in the field.

In the UK there is also evidence that some winter wheat varieties perform better than others in terms of yield in the presence of take-all. This is evident in the UK Recommended List (RL) 1st and 2nd wheat yield trials (www.hgca.com). As mentioned earlier the Recommended List varieties grown in these trials typically yield 1 to 1.5 tonnes/ha less in the second wheat trials than first wheat trials. This reduction in yield between first and second wheats is primarily thought to be due to take-all. Some varieties perform relatively well as the second wheat in the rotation compared with others providing evidence of an interaction between variety and rotational position and potentially differences in tolerance to take-all. RL trials are however carried out on different sites, confounding the results as yield typically has large variety x site interactions. Within the Wheat Genetic Improvement Network (WGIN) programme, Bayles et al. (2007) carried out a study on variety x rotational position interactions with first and second wheat trials on the same site. They found that there were not large differences in take-all root infection of varieties, but that some varieties were consistently better second wheats in terms of yield when tested on the same site in first

and second wheat trials. They attributed this to differences in take-all tolerance between the varieties.

Evidence from the studies described above shows that there are no wheat varieties with a high degree of resistance to take-all. However, small but real differences have been demonstrated. Overall, it is likely that differences in resistance between wheat varieties will not be attributable to a single locus but rather resistance will be polygenic in nature and a combination of different sources of partial resistance would be needed for plant breeding purposes to increase the resistance of wheat.

In contrast to the generally small differences in susceptibility of wheat varieties to take-all there was one UK report in the 1980s that indicated that wheat varieties may differ in the extent to which they encourage inoculum of the take-all fungus to build-up during a first wheat crop (Widdowson et al., 1985). This was investigated more recently as part of the WGIN programme and in the initial year of my PhD, described in the introduction to Chapter 3. Briefly, there were consistent differences detected between a wider 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). This could reduce the risk of severe take-all in a following second wheat crop.

1.7.5. Difficulties assessing wheat germplasm for resistance to take-all

It is hard to compare directly some studies when resistance is scored in different ways. Some measurements such as necrotic root discolouration have been reported as difficult to assess (Penrose, 1992). This could lead to inaccurate/imprecise measurements that could mask differences between varieties. Despite potential difficulties most researchers have measured the amount of root discoloration to assess pathogen growth in different hosts. The amount of root discolouration is normally expressed as a proportion of the total root system (% roots infected). This is a convenient method to use but it could allow differences in take-all infection to be confounded by the rooting density of different hosts.

In laboratory studies often high levels of artificial inoculation have been used over limited time periods. This makes it hard to demonstrate the practical use of any resistance in the field. It may be more productive to look for resistance under moderate disease pressure rather than heavy inoculation as at high disease pressures resistant material will be harder to identify and resistance less likely to be expressed (Scott, 1981).

Possible host genotype-pathogen genotype interactions could complicate the detection of resistance in the field. The different ratios of genotypic sub-populations could mask detection of resistance in the field or between different sites, perhaps contributing to inconsistencies between published results. Also, both pathogen genotype and host genotype probably interact with the environment so that the situation is complicated further. Other confounding effects on take-all severity in the field include seed source and seed weight (Penrose, 1987a).

1.8. Aims and Objectives

The purpose of the PhD project was to identify and characterise novel sources of resistance to the take-all fungus.

The first aim of the PhD project was to investigate the take-all inoculum building ability of current National and Recommended List elite wheat varieties. This was carried out by using a soil core bioassay method to gauge the amount of take-all inoculum in the soil after harvest in first wheat field trials. Variety rotational trials were used to investigate the value of this trait in reducing the risk of take-all in second wheat crops. Pedigree and marker analyses were carried out to identify the potential genetic sources of the trait. The results obtained when investigating the first aim, prompted an evaluation of the epidemiology of the take-all inoculum trait.

The second aim of the PhD project was to explore the susceptibility of current National and Recommended List elite wheat varieties and the diploid wheat species, *Triticum monococcum*, to take-all. This was carried out by evaluating the field reaction of wheat material to take-all in third wheat field trials. The susceptibility of wheat varieties to take-all was also explored at the seedling stage using a pot test method previously developed at Rothamsted Research by others in the take-all research group.

CHAPTER 2: GENERAL MATERIALS AND METHODS

This chapter describes the standard procedures used throughout the project, to avoid repetition between chapters. Changes to any of these methods will be specified where appropriate.

2.1. Maintenance of *Ggt* isolates and long term storage

Isolates of *Ggt* were maintained on 9 cm potato dextrose agar (PDA) plates at 4°C for up to 1 year (39g PDA per litre of water; Oxoid, Basingstoke, UK). Isolates were then sub cultured onto fresh PDA plates, incubated at 15°C until mycelium covered the plate (c. 2 weeks) and stored at 4°C for future use.

The long term storage of *Ggt* isolates was in sterile distilled water (SDW) (Boesewinkel, 1976). Two agar plugs (approx. 1 cm²) were taken from each culture on PDA and transferred to 15 ml SDW in a universal bottle. Two bottles were stored for each culture at room temperature in daylight. According to Boesewinkel (1976) a wide variety of fungal plant pathogens can be stored in this way for at least 7 years without losing viability. For the take-all fungus, Hornby et al (1998) report that an isolate collection had been successfully maintained for over 20 years using this method. However, they also describe a loss in pathogenicity after around 1 to 2 years storage for most isolates. During my PhD I also found that the pathogenicity of isolates was low after 3 years of storage in SDW. Pathogenicity has been reported to be restored from *Ggt* agar cultures following infection of a host plant and re-isolation from infected tissue. Long term preservation of *Ggt* isolates is important to document the isolates used in experiments.

2.2. Field trials

Field trials were either established as the 1st wheat in the rotation or the 3rd wheat position in the rotation. First wheat trials were used to study take-all inoculum build-up and were sown in the autumn after either a 1 or 2 year break away from take-all susceptible cereals to reduce take-all inoculum to negligible levels (Chapter 3). Break crops included winter oilseed rape (*Brassica napus*), winter oats (*Avena sativa*) and beans (*Vicia faba*). Two year crop sequence experiments were also set up to begin as the first wheat in the rotation. Third wheat field trials, after two previous winter wheat crops, were used to study the susceptibility of wheat germplasm to take-all root infection at an expected high natural take-all disease pressure (Chapters 6 and 7). The

wheat varieties used in the two years before the trials were established were typically Robigus, Welford, Brompton or Oakley.

Field trials were all set up on the Rothamsted farm (Hertfordshire, UK) on flinty clay loam soil of the Batcombe soil series. Field trial designs were generated by the Rothamsted Research statistician Rodger White. Each trial was given a unique code number. 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. Field trials were regularly inspected throughout the year, to check on establishment and crop development. Growth stage of the crop was recorded at all sampling points using the Zadoks decimal code for growth stages of cereals (Zadoks et al., 1974). Daily rainfall and average maximum temperatures were downloaded from the electronic Rothamsted Archive (e-RA) for all field seasons.

2.2.1. Soil core bioassay

A soil core bioassay (Slope *et al.* 1979) was used to measure the take-all infectivity of the soil after different wheat varieties sown in the first wheat field trials (Chapter 3). The soil core bioassay was taken after harvest to gauge the amount of take-all inoculum that had built up in the soil under the crop and so predict the risk of severe disease developing if a following second wheat crop was sown. The soil core bioassay was also taken during the growth of the first wheat trials to study the epidemiology of take-all inoculum build-up.

Soil cores (5.5 cm diameter by 10 cm deep; 5 cores per plot) were taken in a zig-zag transect across each plot using an auger. Cores were then inverted into plastic drinking cups (11 cm tall with four 4 mm drainage holes in the bottom) which contained a basal layer of 50 cm³ damp sand. The top of each inverted soil core was crumbled and pressed to the sides of the cup. The cores were then transported back to the field laboratory for storage in cold rooms (5°C). Soil cores were processed over the subsequent months. The soil was lightly sprayed with water and ten wheat seeds of the standard bioassay susceptible wheat variety (cv Hereward, RAGT, Cambridge, UK) were placed on the surface of the soil (originally the bottom of the soil core taken in the field). Seeds were covered with a layer of horticultural grit and pots transferred to a controlled environment room for 5 weeks (16 hour day, 70% RH, day/night temperatures 15/10°C, twice weekly watering). After 5 weeks the plants were removed and the roots washed out with water. The roots were assessed for take-all lesions in a white dish under water

and the number of roots and plants infected were recorded. The percentage of plants and roots infected was calculated as a measure of the infectivity of the soil after different wheat varieties grown as a first wheat.

2.2.2. Plant sampling and take-all disease assessment

Plant samples were taken in the spring and/or summer to assess the susceptibility of wheat germplasm to take-all root infections (Chapters 3, 6 and 7). Self-tie string labels were used, with one or more per plot, to label plant samples. The experiment code and plot number was written on each label in waterproof ink. The plot number was also written on the re-enforced ring on the label in case the label was torn or damaged during transportation or processing of the plant samples.

Spring

For spring plant samples the string labels were tied to a cloth bag. Plant samples were dug from five 15-cm lengths of row for each plot and wrapped in a cloth bag. Plant samples were then transported back to the field laboratory, washed free from soil and stored in a cold room (5°C) and assessed for take-all disease as soon as possible. Samples were examined for take-all lesions in a white dish under water. The total number of plants per plot and the number of plants, seminal and crown roots infected with take-all were recorded per plot. The percentage of plants infected and the number of infected seminal, crown and total roots per plant were then calculated (Bateman et al., 2004, Bateman et al., 2008).

Summer

In the summer each string label was tied onto the end of a length of string (white polypropylene twine). Plant samples were dug from 3, 5 or 10 row lengths of 15 or 20 cm per plot depending on experiment and plot size. Individual plant samples from each plot were tied onto the string. When ten samples were taken from each plot, two sets of labels were used per plot, labelled A and B. Plant samples were transported back to the field laboratory, roots washed free from soil, the tops chopped off and the samples air dried in a polytunnel for 4-5 days. The samples were then stored at room temperature (the dried samples can be stored indefinitely in dry conditions) and examined for take-all disease over the winter months.

Stored dried whole plant roots systems were soaked in water for approx. 15-20 minutes and then assessed in a white dish under water and scored for take-all to calculate a take-

all index (TAI) (Bateman et al., 2004). The proportion of roots infected was estimated and graded slight 1 (1-10% roots infected), slight 2 (11-25%), moderate 1 (26-50%), moderate 2 (51-75%) and severe (more than 75%). From this a take-all index was 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); divide by the number of categories (5); maximum TAI 100. By grading whole plant systems from plot samples by the proportion of roots affected by take-all into categories (slight 1 to severe) the take-all index assesses both the incidence and severity of take-all. The take-all index has therefore been previously described as a measure of take-all intensity (Bateman et al., 2008).

2.3. Pot test method

The pot test method was used to evaluate wheat germplasm for resistance to take-all at the seedling stage in Chapters 6 and 7. This pot assay method was first established at Rothamsted to test the pathogenicity of take-all isolates to wheat and rye seedlings (Gutteridge et al., 1993). The assay originally used a silver sand-coarse grit mixture in the pots. A modified version of this pathogenicity test using take-all free soil has since been developed at Rothamsted to test the efficacy of fungicides (R.J. Gutteridge, 2009, personal communication). This pot test protocol developed at Rothamsted uses field soil collected from take-all free fields (fields not sown with cereals) and artificial inoculum addition to assess the infection of seedlings with take-all.

2.3.1. Preparing *Ggt* inoculum

Inoculum was prepared by first filling 500 ml conical flasks with 100 g silver sand, 3 g maize meal (Polenta) and 10 ml of distilled water. Flasks were autoclaved twice, with 48 hours between autoclaving. Two flasks per individual *Ggt* isolate were prepared. The flasks were inoculated with agar discs (6-mm diameter, cut with a cork borer) from fungal cultures on PDA, adding three discs per flask and using one isolate per flask. The sand/maize meal cultures were incubated at room temperature for 5-7 weeks, with shaking once a week for even colonisation. Sand/maize meal inoculum of different isolates was then added together in sterilised 1000 ml conical flasks to prepare mixtures of isolates for the pot test (see Chapter 5, page 142, for description of isolate characterisation and selection of isolates for pot tests). Flasks of sand/maize meal inoculum were stored at 4°C until use.

2.3.2. The pot test method

Take-all free soil was collected from fields at Rothamsted that had not been sown with cereals. Large stones were removed and the soil was crumbled and stored in buckets at room temperature. Buckets of soil were mixed together before use in the pot assay. A mixture of 250 g take-all free soil and 50 g dilute artificial sand/maizemeal inoculum (mixed in a plastic bag) was transferred into an 11-cm-tall plastic cup which contains a basal layer of 50 cm³ damp sand over four 3-mm-diameter drainage holes in the cup. The dilute artificial inoculum was prepared by mixing the sand/maizemeal inoculum with silver sand (normally at ~ 1:250 dilution of sand/maizemeal inoculum to silver sand, exact dilution calculated from soil calibration tests- see section 2.3.3. below). Ten seeds were then placed on the soil surface and covered with a thin layer of horticultural grit. Five replicates were set up per variety treatment. A control treatment without addition of *Ggt* sand/maizemeal was set up with the standard winter wheat pot test variety Hereward to ensure the soil used was free from take-all. All pots were then gently watered and placed in a controlled environment room in a randomised design (16 hour day, 70% RH, day/night temperatures 15/10°C, twice weekly watering). After 5 weeks the plants were removed and their roots washed out with water before disease assessment in a white dish under water. The total number of plants and roots and the number of plants and roots infected with take-all were recorded. The percentage of plants and roots infected was then calculated.

2.3.3. Soil calibration

The soil calibration was set up in the same way as the standard pot test above but with different dilutions of sand/maizemeal to silver sand for the dilute artificial inoculum (1:50, 1:100, 1:150, 1:200, 1:250, 1:300 and 1:350). The soil calibration test was carried out with our standard susceptible wheat variety, Hereward (RAGT, Cambridge, UK). The aim was to achieve around 50% roots infected in the bioassay plants. This was to ensure the inoculum level was not too high so that good discrimination between genotypes could be achieved if differences in susceptibility to take-all were present.

2.4. Statistics

All data was analysed using Genstat (VSNI, Hemel Hempstead, UK)(Payne et al., 2009). Spearman's rank was used to assess associations between different disease measurements. Percentage disease data was always transformed using the logit transformation before further analysis by REML or ANOVA, to ensure equal variance.

Significant effects were supposed when $p \leq 0.05$. Specific analyses are described in each chapter.

CHAPTER 3: FIELD EVALUATION OF THE TAKE-ALL INOCULUM BUILD-UP (TAB) TRAIT

3.1. Introduction

Historically, when there was not a direct method to quantify *Ggt* DNA in the soil, a soil bioassay method (Slope et al., 1979) has been widely used as a way to gauge the amount of biologically active take-all inoculum in the soil (Hornby et al., 1998). The soil bioassay uses bait wheat plants to measure the infectivity of the soil (Figure 3.1; see Chapter 2 for full soil bioassay method). In the 1950s and 1960s the National Agricultural Advisory Service used seedling infection in soil bioassays to predict fields at risk from take-all and so recommend to farmers fields that were or were not suitable for growing susceptible cereals (Hornby, 1978). By the end of the 1960s this method was no longer used as results were frequently unreliable. However, data collected over many years at Rothamsted suggests that if over 20% of roots are infected in the soil bioassay after harvest of a first wheat crop there is a high risk of severe take-all for a following susceptible cereal crop (R.J. Gutteridge, unpublished data; Hornby et al. 1998). In second and subsequent susceptible cereal crops there is not a good correlation between seedling infection in soil bioassays and disease risk, suggesting that the soil bioassay is only useful to identify risk during the take-all inoculum build-up phase in a first susceptible cereal crop. More recently Gutteridge et al. (2008) confirmed that a good relationship exists between the percentage roots infected in the soil bioassay after harvest of a first wheat crop and take-all severity in a following second wheat crop. Climatic conditions also influence the severity of take-all in a second wheat crop and damaging take-all years have been linked to warm, dull springs and warm but dry summers (Hornby et al., 1998). Although perhaps too labour intensive and time consuming for commercial use the soil bioassay is an important experimental tool. For many years the soil bioassay has been used to measure inoculum in the soil and so compare infested soils, predict the risk of epidemics developing and characterise changes in soil populations of *Ggt* associated with Take All Decline (TAD) (Hornby, 1981).



Figure 3.1. Soil core bioassay method (Slope et al., 1979)(see Chapter 2 for full details of soil bioassay method). Soil cores (at least five per plot) are taken after harvest of a first wheat crop and upturned into plastic cups. Ten wheat seeds of a standard wheat variety (Hereward) are placed on the surface of the soil, covered with horticultural grit and the cups placed in a CE room for 5 weeks (day/night temperatures 15/10 °C). After five weeks the wheat seedlings are washed free of soil and assessed for take-all lesions. The percentage of roots infected is calculated.

In the 1990s work in Australia focussed on detecting and quantifying take-all DNA in the soil (Keller et al., 1995, Herdina et al., 1996), and predicting take-all disease risk based on *Ggt* DNA levels (Herdina et al., 1997, Herdina & Roget, 2000). This work has since been used in Australia for the development of a commercially available DNA based soil pathogen test (Ophel-Keller et al., 2008). This test, called PreDicta B, is used to predict fields at risk from take-all (and other soil-borne pathogens) and so help farmers plan their cropping strategy to reduce losses due to disease. Soil *Ggt* DNA levels were related to soil bioassay results and risk categories developed based on the relationship between soil bioassay results and take-all disease development (Herdina & Roget, 2000, Ophel-Keller et al., 2008). The risk of take-all for the test is divided into four categories of below detectable limit, low, medium and high risk based on *Ggt* DNA per gram of soil. The quantification of *Ggt* DNA using this new method has been used

experimentally in New Zealand to explore the effect of wheat volunteers on inoculum levels during a break crop (Bithell et al., 2011b). Bithell et al. (2012) have also recently evaluated the method to predict take-all risk in commercial wheat fields in New Zealand, reporting that a set of three risk categories better separate the risk of take-all for different fields under conditions in New Zealand. Comparisons between quantified *Ggt* DNA and infectivity in the soil bioassay have shown a generally good relationship between the two methods (Gutteridge et al., 2008, Bithell et al., 2009). This supports the use of the soil bioassay as a gauge of take-all inoculum levels in the soil. However, as stated in McMillan et al. (2011) it is acknowledged that other soil chemical, physical and biological factors could influence soil infectivity making soil more conducive or suppressive to disease, regardless of the actual amount of take-all inoculum.

Earlier work in the 1980s using the soil core bioassay suggested that there were differences in the amount of take-all inoculum left in the soil after two different wheat varieties (Avalon and Norman) when they were grown as a first wheat (Widdowson et al., 1985). Widdowson et al. (1985) also reported that disease severity in the following crop was related to the amount of take-all inoculum gauged using the soil bioassay after the first wheat crop.

This theory of differential take-all inoculum build-up between wheat varieties grown in a first wheat situation has been further tested in first wheat field trials as part of the Wheat Genetic Improvement Network (WGIN) programme. In the initial year of my PhD I helped collect the 5th year of data from these trials using the soil bioassay method and these data have since been published in Plant Pathology (McMillan et al. 2011). This study demonstrated that consistent differences do exist between wheat varieties in their ability to build-up take-all inoculum in the soil when grown as first wheat crops. We called this the **take-all inoculum build-up (TAB) trait**.

The majority of the wheat varieties used in the WGIN field trials were not currently grown and did not feature on the HGCA recommended winter wheat variety lists (RL) of the time. However, some of the wheat varieties tested (for example Cadenza, Claire, Avalon) were previously recommended varieties and have been widely used in wheat breeding programmes so their pedigrees are represented in current commercial varieties (see Chapter 4 for pedigree analysis information). One of the main objectives of my PhD study was to evaluate a wider range of current commercial elite wheat varieties for the TAB trait. In this study a series of 3 consecutive first wheat field trials were set up to evaluate the TAB trait in 45 elite winter wheat varieties. Varieties from the WGIN

trials (namely Avalon, Cadenza and Hereward) were included as controls to compare with the TAB phenotype of current RL varieties. Soil inoculum epidemiological studies on selected varieties within the trials were carried out as part of HGCA funded summer bursary projects. This was to examine the time course of take-all inoculum build-up and identify when varietal differences can be detected in the field during the key months of inoculum build-up from April/May to harvest.

In order to identify whether the low TAB phenotype could be of practical use to reduce the risk of take-all disease, rotational studies have been set up as part of the on-going WGIN programme. The aim of the rotational studies was to measure take-all severity and yields in second wheat crops after a low (Cadenza) or high take-all inoculum building (Hereward) first wheat variety. Eight different wheat varieties were chosen in the second year of the rotation to explore how different combinations of first and second wheat varieties affect the take-all epidemic. I have helped to assess samples and analyse results from these rotational studies. Results from the first two of these rotational studies (harvest years 2009&2010 and 2010&2011), carried out during my PhD, are reported here.

3.2. Materials and Methods

Procedures for evaluating take-all inoculum build-up in the field are described in Chapter 2: General Materials and Methods. Details of individual field experiments are given in Table 3.1 and field trial plans in Appendices 3.1 and 3.2.

Table 3.1. Details of the field experiments used to evaluate the TAB trait.

Harvest year (Rothamsted field trial code)	Rothamsted Field	Previous cropping history		Sowing date	Plot size (m)	Date harvested
		Preceding year	2 years previous			
Elite winter wheat trials						
2009 (09/R/WW/916)	New Zealand	Winter rape	Winter wheat	09/10/08	10 x 2	13/08/09
2010 (10/R/WW/1032)	Great Knott 1	Winter rape	Winter wheat	15-16/10/09	9 x 3	16/08/10
2011 (11/R/WW/1115)	Pastures	Winter rape	Spring barley	09/10/10	10 x 3	12/08/11
Rotation trial 1						
Year 1: 2009 (09/R/CS/688)	Great Knott 3	Winter oats	Winter wheat	10/10/08	12 x 82	28/08/09
Year 2: 2010 (10/R/CS/688)	Great Knott 3	Winter wheat	Winter oats	09/10/09	10 x 3	06/08/10
Rotation trial 2						
Year 1: 2010 (10/R/CS/706)	Great Knott 1	Winter rape	Winter wheat	24/09/09	12 x 82	05/09/10
Year 2: 2011 (11/R/CS/706)	Great Knott 1	Winter wheat	Winter rape	10/10/10	10 x 3	12/08/11

3.2.1. Elite winter wheat varieties and inoculum build-up 2009-2011

Three winter wheat field trials, in the harvest years of 2009, 2010 and 2011 were set up to measure the inoculum building ability of current elite winter wheat varieties (Table 3.1). All trials were sown as first wheat crops after winter oilseed rape and consisted of four replicates of 45 previous, current or candidate HGCA Recommended List winter wheat varieties. Due to the large number of varieties per replicate it was expected that there would be great variability in background soil conditions and take-all inoculum build-up even within a block. This is particularly important when studying take-all disease which has a notoriously patchy distribution in the field. To help control the variability the 45 varieties within each of the four large replicate blocks were further grouped into smaller incomplete sub-blocks. Upon analysis this allows more of the residual variation within the trial to be removed from the estimates of varietal means. Variety means are then formed from weighted sums of the variety values from the sub-blocks. This basic design with the additional sub-blocking within whole blocks is an alpha design. The designs were all generated by Rodger White using CycDesigN (VSN International Limited, Hemel Hempstead, UK).

The same 45 winter wheat varieties were used in all years apart from three replacements in the second trial year and one additional replacement in the third year; this was due to limited seed availability of the original varieties (Table 3.2). Thirty-five out of the forty-nine wheat varieties tested over the three years had not been previously assessed for the TAB trait within the WGIN field trials. Varieties previously tested in the WGIN diversity field trials are shown in bold text in Table 3.2. Trials were sown in the autumn at a seed rate of 350 seeds/m². Yields were taken from each plot by the Rothamsted farm.

The soil core bioassay (8 soil cores per plot), described in Chapter 2, was used to measure the infectivity of the soil after harvest. Sampling took place over several days for the elite winter wheat trials due to the large number of cores being taken. However, sampling of any one of the four blocks in a trial was always carried out within a single day. Percentage disease data was always transformed using the logit transformation, to ensure equal variance. Transformed data was analysed by Rodger White using REML to incorporate the sub-blocking structure.

Table 3.2. Winter wheat varieties used in field experiments 09/R/WW/916, 10/R/WW/1032 and 11/R/WW/1115.

Code	Variety ¹	Years in trials	Nabim group ²	Date first listed ³	Breeder ⁴	Parentage ⁵
Al	Alchemy	3	4	2006	Nick	Claire x (Consort x Woodstock)
Av	Avalon	3	1	1980	PBI	TJB 30/148 x TL 365A/34
Bn	Bantam	3	4	NR (2008)	Nick	Xi19 x NSL WW35
Bt	Battalion	3	2	2007	RAGT	98ST08 x Aardvark
Br	Brompton	3	4	2005	Els	CWW 92.1 x Caxton
Ca	Cadenza ⁶	3	2	1994	CPB	Axona x Tonic
Cs	Cassius	3	4	2009	Nick	Claire x (NSL WW24 x Wizard)
Cl	Claire	3	3	1999	Nick	Wasp x Flame
Cn	Conqueror	3	4	2010	KWS	Robigus x Equinox
Cr	Cordiale	3	2	2004	CPB	(Reaper x Cadenza) x Malacca
Du	Duxford	3	4	2008	NFC	Solstice x Scorpion 25
Ed	Edmunds	3	3	NR (2009)	Nick	Deben x Napier
Ei	Einstein	3	2	2003	Nick	(NHC49 x UK Yield Bulk) x (Haven x Clarion)
Ga	Gallant	3	1	2009	Syn	(Malacca x Charger) x Xi19
Gl	Gladiator	3	4	2004	Mon	Falstaff x Shannon
Gw	Glasgow	2	4	2005	SU	(Ritmo x SUR 90-2666) x SUR 91-11658
Gr	Grafton	3	4	2009	KWS	Cordiale x CPBT W97
Hf	Hereford	1	4	NR (2007)	Sej	Solist x Deben
Hw	Hereward	3	1	1991	PBI	Norman 'sib' x Disponent
Hu	Humber	3	4	2007	CPB	Anglo x Krakatoa
Hy	Hyperion	2	4	2006	Nick	Aardvark x (Consort x Woodstock)
In	Invicta	3	3	2010	Nick	NSLWW48 x Robigus
Is	Istabraq	3	4	2004	Nick	Consort x Claire
Jb	JB Diego	3	4	2008	Breun	3351b x Stru2374
Ke	Ketchum	3	2	2009	Syn	Solstice x Xi19
Kg	Kingdom	1	2	2010	Syn	Cordiale x Xi19
Ki	Kipling	3	4	NR (2006)	Depr	Hunter x 9205-4

Table 3.2. Continued

Code	Variety ¹	Years in trials	Nabim group ²	Date first listed ³	Breeder ⁴	Parentage ⁵
Le	Lear	3	4	NR (2008)	Nick	Robigus x Nijinsky
MI	Malacca	3	1	1999	CPB	Riband x (Rendevouz) x Apostle
Mw	Maris Widgeon	2	1	1964	PBI	Holdfast x Cappelle-Desprez
Mr	Marksman	3	2	2008	RAGT	98ST08 x Aardvark
Ms	Mascot	3	1	2006	RAGT	Reaper x Rialto
Mn	Monty	1	4	NR (2007)	Syn	Robigus x NFC10035
Oa	Oakley	3	4	2007	CPB	(Aardvark 'sib' x Robigus) x Access
Pn	Panorama	3	2	2009	Nick	(Xi19 x Solstice) x Solstice
Pa	Paragon ⁷	2	1	1999	PBI	CSW 1724/19/6/68 x (Axona x Tonic)
Qp	Qplus	3	2	2009	Nick	Solstice x Robigus
Ri	Riband	3	4	1989	PBI	Norman x (Maris Huntsman x TW161)
Ro	Robigus	3	3	2003	CPB	Z836 x 1366
Sc	Scout	3	3	2009	Sen	Z435 x Deben
Se	Sherborne	3	4	NR (2007)	KWS	Aardvark sib x Biscay
Sh	Shogun	3	4	NR (2008)	RAGT	Mallet x Whistler
Si	Soissons	3	2	1995	Depr	Jena x HN 35
So	Solstice	3	1	2002	Adv	Vivant x Rialto
Vi	Viscount	3	4	2009	KWS	Robigus x Canterbury
Wa	Walpole	1	2	NR (2008)	Nick	Xi19 x Solstice
We	Welford	3	4	2004	Els	CWW 92/1 x FD92054
Xi	Xi19	3	1	2002	Adv	(Cadenza x Rialto) x Cadenza
Ze	Zebedee	3	3	2007	Nick	Claire x Nelson

¹ Varieties in bold have been previously tested for the TAB trait in WGIN field trials (McMillan et al., 2011).

² Nabim groups; Group 1 = quality breadmaking wheats, Group 2 = breadmaking potential wheats, Group 3 = biscuit wheats, Group 4 = feed wheats.

³ Date first listed in UK Recommended List (RL). NR = Not recommended (first candidate year).

⁴Original breeder in year first listed. Adv, Advanta Seeds UK; Breun, Saatzucht Josef Breun, Germany; CPB, CPB Twyford; Depr, Maison Florimond Desprez, France; Els, Elsoms Seeds; KWS, KWS UK; Mon, Monsanto; NFC, New Farm Crops; Nick, Nickersons; PBI, Plant Breeding Institute; RAGT, RAGT Seeds; Sej, Sejet, Denmark; Sen, Senova; SU, Saaten Union UK; Syn, Syngenta Seeds.

⁵Parentage information obtained from breeder websites, archive HGCA Recommended Lists and NIAB association pocket guides to varieties of cereals, oilseeds and pulses.

⁶Cadenza = facultative spring wheat.

⁷Paragon = spring wheat.

Epidemiology studies on the build-up of take-all inoculum were carried out on six selected varieties (Table 3.3). The six wheat varieties were selected for epidemiology studies due to their consistent performance in the previous first wheat WGIN field trials based on the overall amount of take-all inoculum built-up by harvest (McMillan et al., 2011). These varieties were the consistent low builders Cadenza, Cordiale and Xi19, the moderate take-all inoculum builder Riband, and the higher take-all inoculum building varieties Avalon and Hereward. In 2009 and 2010 this work was part of HGCA funded summer bursary projects with students James Bruce (2009) and Nicola Phillips (2010). Five soil cores were taken per plot for each of the six varieties at monthly intervals from March or April through to harvest when the final soil cores were taken from all plots (Table 3.4). A cross-season analysis was carried out using a repeated measurements ANOVA in Genstat (Payne et al., 2009).

Table 3.3. Varieties selected for epidemiology studies in field trials 09/R/WW/916, 10/R/WW/1032 and 11/R/WW/1115.

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

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

Plant samples for take-all disease assessment (Chapter 2) were taken in the summer between GS 73-83 for all varieties in the three field trials (Table 3.4) Whole plant samples were dug from five 20 cm lengths of row per plot in a zig-zag transect. Disease data was analysed by Rodger White using REML.

Additional crop measurements were taken in the second and third field trial years (Table 3.4). In the second field trial (10/R/WW/1032) ear emergence, leaf rolling and leaf senescence were recorded. In the third field trial (11/R/WW/1115) leaf senescence, soil pH and in-field soil moisture and soil temperature were recorded. Spearman's rank correlation was used on untransformed data to assess the strength of association between disease data, yields and field phenotype observations. Ear emergence, leaf rolling and leaf senescence were recorded by looking at each plot lengthwise and assessing what state the majority of the plants were in the central area of the plot. The

angle of the sun can affect the way plants are viewed; to minimise this effect all assessments were made in the afternoon and the plots assessed by walking the field trials in the same direction each time. Ear emergence was recorded for each plot roughly twice a week from GS 41 (Flag leaf sheath extending) to GS 59 (Inflorescence completed). Leaf rolling in response to the dry weather in summer 2010 was recorded for each plot as the majority of plants either rolled (R) or not-rolled (NR). Leaf senescence was recorded using a leaf senescence key (Appendix 3.3) received from Simon Orford at the John Innes Centre (JIC, Norwich, UK). Leaf senescence was assessed twice in July during the 2010 field trial and three times during the 2011 field trial. Plots were assessed on a scale of 1 to 10 (10 = maximum senescence). Plots were re-checked regularly to ensure that scoring was accurate.

Samples for soil pH analysis were taken for two selected varieties (Hereward and Cadenza; 4 reps of each) from the 2011 elite winter wheat field trial (11/R/WW/1115). These samples were taken at monthly intervals from April to after harvest at the same time as the epidemiology soil core bioassay. Whole plant samples were dug up from five 15 cm row lengths per plot and placed in buckets. The samples were transported back to the field laboratory where the soil from around the crown/root region was knocked off and crumbled. This soil was sieved to 2 mm and air dried for 1-2 weeks. Then 10 g dried soil was weighed out into 60 ml glass bottles for soil pH analysis by the Rothamsted analytical lab. A cross-season analysis was carried out using a repeated measurements ANOVA.

In May 2011 a soil moisture and temperature sensor (SM300, Delta-T Devices Ltd, Cambridge, UK) and attached data logger (GP1, Delta-T Devices Ltd, Cambridge, UK) were placed within the 2011 elite winter wheat field trial to record in-field soil conditions throughout the key months of inoculum build-up. A small hole was dug using a trowel in plot 141 of the trial and the SM300 sensor pushed into the soil, ensuring good contact of the rods with the soil. The sensor was covered over with soil and the attached data logger left on the soil surface above. The data logger was programmed using DeltaLINK software (version 2.5.1) to record soil moisture and temperature from the sensor at 30 minute intervals. At roughly weekly intervals a laptop was taken into the field and the readings downloaded from the data logger. The sensor and data logger were left in situ in the field until the trial was harvested in August 2011.

Table 3.4. Sampling details of the 1st wheat elite winter wheat variety and take-all inoculum build-up field trials 2009-2011.

Harvest year (field trial code) Varieties sampled	Sampling method/ additional measurements	Units per plot ¹	Month	Date sampled	Growth Stage (GS) ²
2009 (09/R/WW/916)					
Epidemiology study ³ Six varieties	Soil bioassay	5 cores	April	15/04/2009	22/23
	Soil bioassay	5 cores	May	18/05/2009	37
	Soil bioassay	5 cores	June	16/06/2009	60
	Soil bioassay	5 cores	July	10/07/2009 ⁴	75
All 45 varieties	Plant samples	5 x 20 cm	July	13-14/07/2009	75
	Soil bioassay	8 cores	August	25-28/08/2009	After harvest
2010 (10/R/WW/1032)					
Epidemiology study ³ Six varieties	Soil bioassay	5 cores	March	10/03/2010	14
	Soil bioassay	5 cores	April	15/04/2010	25
	Soil bioassay	5 cores	May	19/05/2010	37
	Soil bioassay	5 cores	June	15/06/2010	61
	Soil bioassay	5 cores	July	19/07/2010	75
All 45 varieties	Plant samples	5 x 20 cm	June	29-30/06/2010	73
	Soil bioassay	8 cores	August	19-25/08/2010	After harvest
	Ear emergence	GS	June	2-17/06/2010	41-59
	Leaf rolling	R or NR ⁵	June	29/06/2010	73
	Leaf senescence	1-10 ⁶	July	15&20/07/2010	75
2011 (11/R/WW/1115)					
Epidemiology study ³ Six varieties	Soil bioassay	5 cores	March	17/03/2011	14
	Soil bioassay	5 cores	April	19/04/2011	24
	Soil bioassay	5 cores	June ⁷	07/06/2011	64
	Soil bioassay	5 cores	June	21/06/2011	73
	Soil bioassay	5 cores	July	19/07/2011	83
All 45 varieties	Plant samples	5 x 20 cm	July	14-15/07/2011	83
	Soil bioassay	8 cores	August	16-17/08/2011	After harvest
	Leaf senescence	1-10 ⁶	July	11, 20 & 26/07/2011	83-87
Cadenza and Hereward plots	Soil samples for pH Analysis	5 x 15 cm	April- August	Same dates as soil bioassay	24 to after harvest

¹ Plant sampling units in lengths of row (cm).

² Zadoks decimal code for cereals.

³ Epidemiology studies were carried out on six selected varieties: Avalon, Cadenza, Cordiale, Hereward, Riband and Xi19 (Table 3.3).

⁴ Sampled a week early due to suitable weather conditions/rainfall event.

⁵ R = rolled leaves, NR = not rolled leaves.

⁶ Leaf senescence key- Appendix 3.3.

⁷ Ground was too hard and dry to take soil cores in May; Soil cores taken on June 7th after rainfall.

3.2.2. Rotation experiments

Two 2-year rotation field trials were set up in autumn 2008 and autumn 2009 after winter oats and winter rape, respectively (Table 3.1). In Year 1 the trials consisted of randomised block designs of 4 replicates of the winter wheat varieties Hereward (high TAB variety) and Cadenza (low TAB variety). These were sown as large plots of 12 m x 82 m. In Year 2 each of the large plots of Hereward and Cadenza from the previous year were divided into eight 10 m x 3 m plots and sown with 8 different elite winter wheat varieties. Varieties were chosen to cover a selection of nabim groups, wheat breeding companies and 2nd wheat performance in terms of yield (Table 3.5). Solstice, Xi19 and Hereward have similar first and second wheat performances as a percentage of the treated control yield, while Robigus has generally been considered a ‘bad’ second wheat in terms of yield. Cordiale, Duxford, Einstein and Gallant all yield relatively well in the second wheat position. Trials were sown at a seed rate of 350 seeds/m², but were larger (500 seeds/m²) for the Cadenza plots in the 1st rotation trial (established autumn 2008) due to poor performance in seed germination tests.

After harvest in year 1 the soil core bioassay was taken (Table 3.6). Five soil cores were taken from the location of each of the following year’s (year 2) designated plots (64 plots in total). In year 2 plant samples were taken in both the spring and summer for take-all disease assessments as described in Chapter 2. In the spring whole plant samples were dug from five 15 cm lengths of row and in the summer samples were taken from ten 20 cm lengths of row (Table 3.6). In year 2 yields were taken by the Rothamsted farm from each plot.

In the summer of year 2 of each rotation trial the extent of above ground take-all symptoms was to be recorded. The take-all patch score is assessed by estimating the percentage of each plot area showing prematurely ripened stunted plants caused by take-all (Bateman & Hornby, 1999, Gutteridge et al., 2006, Bateman et al., 2008). However dry weather/drought conditions in the spring/early summer of both 2010 and 2011 caused premature ripening and yellowing of leaves throughout both trials. Take-all patches were not clearly visible under these conditions so were not assessed.

Two-way ANOVAs were used to analyse the main effects and interaction between year 1 ‘source’ variety and year 2 ‘oversow’ variety. In year 2 of the first rotation trial (10/R/CS/688) there was seed spill on plots 48 and 64 so samples were not taken from

these plots and they were treated as missing values in the analysis. In year 2 of the second rotation trial (11/R/CS/706) one sample (03B) was missing.

Table 3.5. Winter wheat varieties chosen for 2nd year of rotation experiments 10/R/CS/688 and 11/R/CS/706.

Variety	Nabim group	Current Breeder	HGCA RL 2009 yields ¹	
			% control	
			Rotational position	
			1 st cereal ²	2 nd and more ³
Cordiale	2	KWS	100	102
Duxford	4	Syngenta	103	108
Einstein	2	Limagrain	100	104
Gallant	1	Syngenta	103	104
Hereward	1	RAGT	89	91
Robigus	3	KWS	102	98
Solstice	1	Limagrain	98	99
Xi19	1	Limagrain	101	102

¹Data from the HGCA Recommended List® for Winter Wheat 2009/2010.

²First cereal treated control 10.6 tonnes/ha.

³Second and more treated control 9.6 tonnes/ha.

Table 3.6. Sampling details of rotation field trials 2009-2011.

Trial		Units		Date	
Harvest year (field trial code)	Sampling method	per plot	Month	sampled	Growth stage
Rotation trial 1					
Year 1: 2009 (09/R/CS/688)	Soil bioassay	5 cores	September	10/09/2009	After harvest
Year 2: 2010 (10/R/CS/688)	Spring plant samples	5 x 15 cm	April	22/04/2010	31
	Summer plant samples	10 x 20 cm	July	14/07/2010	75
	Take-all patch score	% area	Not assessed ¹		
Rotation trial 2					
Year 1: 2010 (10/R/CS/706)	Soil bioassay	5 cores	September	08/09/2010	After harvest
Year 2: 2011 (11/R/CS/706)	Spring plant samples	5 x 15 cm	April	18/04/2011	31
	Summer plant samples ²	10 x 20 cm	July	13/07/2011	81
	Take-all patch score	% area	Not assessed ¹		

¹ Dry weather in the spring/early summer of both 2010 and 2011 caused premature ripening and yellowing of leaves. Take-all patches were not clearly visible under these conditions so were not assessed.

² One sample missing (03B).

3.2.3. Microscopic analysis

In year 1 of the first rotation trial (09/R/CS/688) a large proportion of bait plant roots in the soil core bioassay showed grey discolouration and did not have typical black take-all lesions. This was common in samples throughout the whole field trial. In the 2011 elite winter wheat variety field trial (11/R/WW/1115) there was also evidence of grey roots in the soil bioassay, but not from across the whole field trial site. In both cases the grey roots were viewed under a binocular microscope ($\times 25$ objective, $\times 10$ eyepiece) and swollen cells typical of root infection by *Phialophora graminicola* (anamorph of *G. cylindrosporus*) were seen (Hornby et al., 1998).

3.3. Results

3.3.1. Elite winter wheat varieties and inoculum build-up 2009-2011

Only one year out of the three elite winter wheat variety first wheat trials sampled showed significant differences between varieties in their ability to build-up inoculum of the take-all fungus (2009, $P < 0.001$; 2010 and 2011, $P > 0.4$; Table 3.7). Thirty-three out of the forty-five wheat varieties tested in 2009 had not been previously assessed for the TAB trait in the WGIN field trials (McMillan et al., 2011) and so this represents the first information on this trait across a wider range of the current elite winter wheat varieties. Unfortunately varietal performance could not be confirmed in 2010 or 2011. Unfavourable weather conditions in 2010 and anomalous within field take-all inoculum distribution in 2011 resulted in unsuitable conditions for testing varieties. This meant it was also not possible to carry out a combined year statistical analysis.

Table 3.7. Take-all infectivity of the soil (measured using the soil core bioassay) after harvest of elite winter wheat variety trials sown as first wheat crops, 2009-2011.

Variety ¹	Logit % roots with take-all (back-transformed means)		
	Year		
	2009 ² (09/R/WW/916)	2010 ³ (10/R/WW/1032)	2011 ⁴ (11/R/WW/1115)
Glasgow		-1.51 (4.1)	0.12 (55.5)
Kingdom			-0.94 (12.8)
Maris Widgeon		-1.83 (2.0)	0.16 (57.6)
Paragon		-1.70 (2.7)	-0.01 (48.9)
Malacca	-0.14 (42.8)	-2.71 (0.0)	-0.22 (38.8)
Cordiale	-0.09 (45.1)	-2.55 (0.1)	-1.15 (8.7)
Alchemy	-0.07 (45.9)	-2.12 (0.9)	0.23 (60.6)
Bantam	-0.06 (46.5)	-2.40 (0.3)	-0.84 (15.3)

Table 3.7. Continued

Variety ¹	2009 ² (09/R/WW/916)	2010 ³ (10/R/WW/1032)	2011 ⁴ (11/R/WW/1115)
Invicta	0.06 (52.3)	-2.31 (0.5)	-0.35 (32.9)
Hereford	0.07 (53.1)		
Zebedee	0.07 (53.1)	-2.71 (0.0)	-0.28 (35.7)
Panorama	0.09 (54.0)	-2.10 (1.0)	-0.39 (30.9)
Grafton	0.09 (54.2)	-2.11 (1.0)	-0.91 (13.5)
Solstice	0.10 (54.5)	-2.89 (0.0)	0.41 (69.1)
Gallant	0.11 (55.1)	-2.46 (0.2)	-0.50 (26.5)
Gladiator	0.11 (55.2)	-2.37 (0.4)	-0.03 (48.2)
Xi19	0.12 (55.7)	-2.55 (0.1)	-0.08 (45.5)
Cadenza	0.13 (55.8)	-2.80 (0.0)	0.02 (50.4)
Lear	0.14 (56.3)	-2.01 (1.3)	-0.27 (36.1)
Cassius	0.16 (57.5)	-2.48 (0.2)	-0.79 (16.6)
Claire	0.17 (58.1)	-2.83 (0.0)	-0.76 (17.5)
Ketchum	0.19 (59.1)	-2.26 (0.6)	-0.37 (31.6)
Edmunds	0.20 (59.6)	-2.51 (0.2)	-0.19 (40.3)
Battalion	0.21 (59.9)	-2.52 (0.1)	0.05 (52.1)
Sherborne	0.21 (60.0)	-2.68 (0.0)	-0.52 (25.7)
Humber	0.22 (60.2)	-2.05 (1.1)	0.03 (50.9)
Oakley	0.23 (60.8)	-2.51 (0.2)	-0.38 (31.2)
Kipling	0.24 (61.5)	-2.09 (1.0)	-0.15 (42.0)
Marksman	0.26 (62.3)	-2.97 (0.0)	-0.23 (38.2)
Soissons	0.32 (65.0)	-2.28 (0.5)	0.21 (60.0)
Einstein	0.33 (65.3)	-1.97 (1.4)	-0.68 (19.9)
Scout	0.34 (66.1)	-2.03 (1.2)	-0.10 (44.4)
Hyperion	0.39 (67.9)	-2.20 (0.7)	
Conqueror	0.39 (68.1)	-2.51 (0.2)	0.42 (69.2)
Robigus	0.39 (68.1)	-2.88 (0.0)	-0.18 (40.7)
Shogun	0.39 (68.1)	-2.47 (0.2)	0.02 (50.5)
Walpole	0.40 (68.4)		
Mascot	0.41 (68.8)	-2.49 (0.2)	-0.23 (38.1)
JB Diego	0.41 (69.1)	-2.38 (0.4)	-0.45 (28.5)
Riband	0.41 (69.1)	-2.55 (0.1)	-0.21 (39.2)
Istabraq	0.43 (69.8)	-2.10 (1.0)	-0.36 (32.1)
Avalon	0.47 (71.4)	-2.23 (0.7)	-0.68 (19.8)
Brompton	0.48 (71.7)	-2.23 (0.6)	-0.63 (21.8)
Hereward	0.48 (71.8)	-2.35 (0.4)	0.09 (54.0)
Qplus	0.50 (72.4)	-2.74 (0.0)	-0.82 (15.7)
Monty	0.50 (72.7)		
Welford	0.52 (73.5)	-1.98 (1.4)	-0.29 (35.4)
Viscount	0.55 (74.5)	-2.04 (1.2)	0.08 (53.7)
Duxford	0.61 (76.8)	-2.44 (0.3)	-1.26 (7.0)

Table 3.7. Continued

d.f.	44	44	44
SED (Average)	0.16	0.48	0.56
Wald statistic	121.58	40.52	44.53
F Probability	<0.001	0.614	0.465
Grand mean	0.26 (61.7)	-2.35 (0.6)	-0.30 (36.9)

¹ **Bold** = varieties previously tested for the TAB trait in WGIN field trials (McMillan et al., 2011).

² Varieties are sorted in order of 2009 TAB score. Variety performance could not be adequately explored in 2010 or 2011.

³ In 2010 TAB was restricted across the whole trial site due to dry weather.

⁴ In 2011 the presence of *Phialophora graminicola* resulted in uneven take-all inoculum build-up across the field trial site.

Within the WGIN field trials Cadenza, Cordiale and Xi19 were consistently among the lowest inoculum building varieties while Avalon and Hereward represented the higher inoculum builders (McMillan et al., 2011). In the 2009 field trial these five varieties performed as expected (Table 3.7). In addition there were nine previously untested varieties with lower inoculum building scores than Cadenza and Xi19 and five varieties with higher inoculum building scores than both Avalon and Hereward. Not all of the previously tested varieties performed as expected based on the results of the WGIN diversity field trials. Within the WGIN trials Riband was a low to moderate TAB variety but in the 2009 trial Riband was at the higher end of the TAB scale, closer to the high builders Avalon and Hereward than the low builders Cadenza and Xi19 (Table 3.7). The lowest TAB variety in 2009 was Malacca but within the WGIN field trials Malacca was generally one of the highest building varieties. These findings emphasize the need for multiple years of trials when assessing the TAB trait.

There was negligible take-all disease found on the roots of the plant samples from all varieties in the 2009 first wheat field trial with a mean take-all index (TAI) of 1.02 across the whole trial (scale: 0-100) (Table 3.8). Differences between varieties were not significant ($P = 0.427$). In contrast plant samples taken from the same 45 varieties grown in a 3rd wheat field trial in the same year had an average take-all index of 74.07 (see Chapter 6). There was no strong correlation found between the percentage roots infected of the bait plants in the soil core bioassay and the TAI of plant samples per

field plot or between the mean values for each variety (Per plot, Spearman's rank [Rs] = 0.07, P = 0.35, n = 180; Per variety, Rs = -0.13, P = 0.41, n = 45). Yields are inherently different between varieties but a slight significant negative correlation between yield and TAB can be detected when analysing observations per plot (Rs = -0.28, P < 0.001, n = 180, Figure 3.2). This association between TAB and yield was weaker and not significant when the mean values for each variety were analysed (Rs = -0.18, P = 0.24, n = 45).

Table 3.8. The incidence and severity of take-all disease on plant roots from the first wheat elite winter wheat variety field trials, 2009-2011.

Variety	Take-all Index (TAI, 0-100)		
	Year		
	2009 (09/R/WW/916)	2010 (10/R/WW/1032)	2011 (11/R/WW/1115)
Alchemy	1.29	0.35	17.88
Avalon	0.42	1.54	9.81
Bantam	0.92	0.14	5.98
Battalion	0.86	1.75	8.05
Brompton	0.52	0.34	7.84
Cadenza	1.22	0.86	4.99
Cassius	0.73	0.66	3.54
Claire	1.14	0.16	11.83
Conqueror	1.91	0.15	26.51
Cordiale	2.42	0.67	4.19
Duxford	1.19	0.79	4.47
Edmunds	0.97	1.26	12.91
Einstein	2.26	-0.16	6.77
Gallant	0.65	1.14	2.89
Gladiator	0.94	0.80	10.17
Glasgow		1.89	4.20
Grafton	0.72	-0.06	5.79
Hereford	0.95		
Hereward	2.57	0.54	8.37
Humber	0.41	0.44	10.35
Hyperion	1.00	1.00	
Invicta	0.65	-0.09	5.66
Istabraq	0.17	0.45	4.38
JB Diego	1.00	0.85	4.02
Ketchum	0.00	0.44	4.65
Kingdom			6.93
Kipling	2.16	0.28	4.19
Lear	0.65	0.91	3.05

Table 3.8. Continued

Variety	2009 (09/R/WW/916)	2010 (10/R/WW/1032)	2011 (11/R/WW/1115)
Malacca	0.79	0.23	8.70
Maris Widgeon		3.01	2.32
Marksman	0.13	0.38	14.15
Mascot	0.95	1.94	7.46
Monty	0.21		
Oakley	-0.01	0.78	6.48
Panorama	1.29	2.16	1.70
Paragon		2.68	3.25
Qplus	1.34	0.59	11.38
Riband	0.70	2.05	4.63
Robigus	0.97	0.20	13.94
Scout	0.65	0.94	6.34
Sherborne	1.28	0.20	4.92
Shogun	0.13	1.06	6.36
Soissons	0.64	0.84	14.72
Solstice	2.12	0.59	7.36
Viscount	1.40	1.44	10.68
Walpole	0.00		
Welford	1.48	1.01	3.02
Xi19	1.84	0.89	9.00
Zebedee	2.15	1.21	5.62
d.f.	44	44	44
SED (Average)	0.94	0.91	5.26
Wald statistic	45.79	53.97	69.73
F Probability	0.427	0.192	0.026
Grand mean	1.02	0.87	7.59

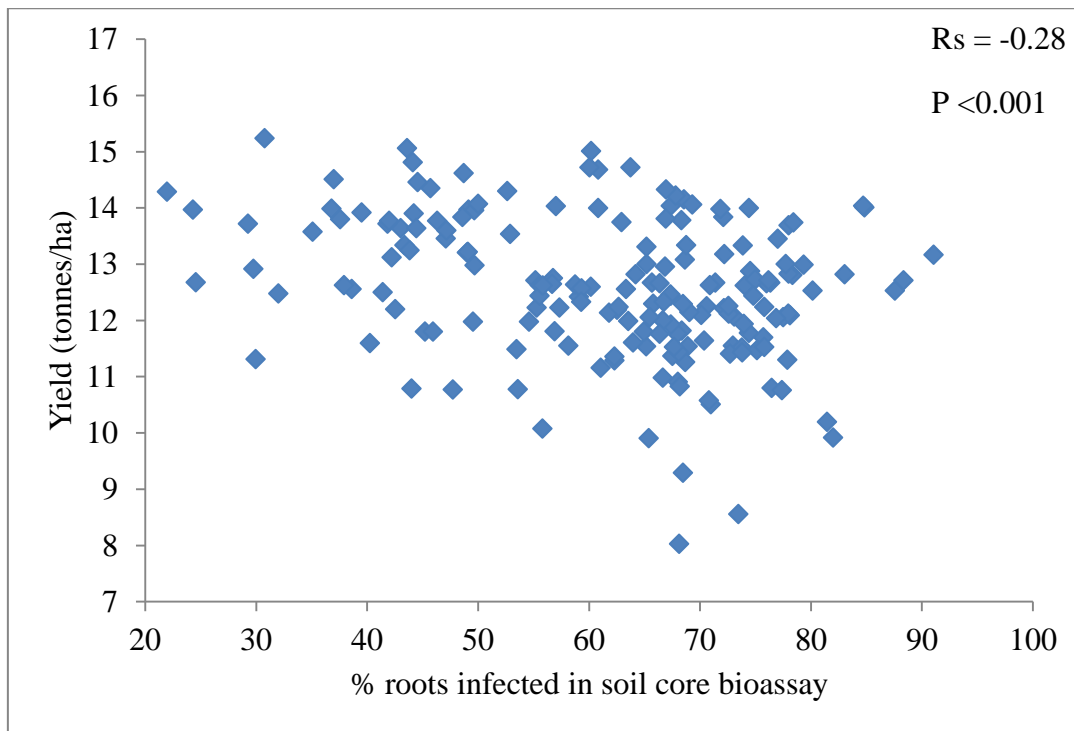


Figure 3.2. Per plot correlation between the percentage roots infected on bait plants in the soil core bioassay and yields in the 2009 first wheat field trial (09/R/WW/916).

In 2010 significant levels of take-all inoculum failed to develop over the whole field trial (back-transformed grand mean across whole trial: 0.6% roots infected; Table 3.7). In the months of April, June and July of 2010 there was half the total rainfall than in the previous year, which probably restricted the build-up of inoculum (see section 3.3.3). Leaf rolling as an expression of drought stress due to the dry weather was recorded for all plots in June; thirty-four of the forty-five varieties displayed rolled leaves in one or more of the four replicates. There was very little take-all identified on the roots of plant samples (Mean TAI across whole trial: 0.87; Table 3.8). A weak positive correlation was detected between the percent roots infected in the soil core bioassay and the TAI of plant samples (Per plot, $R_s = 0.24$, $P < 0.01$, $n = 180$, Figure 3.3; Per variety, $R_s = 0.27$, $P = 0.07$, $n = 45$). There was no significant association between take-all inoculum build-up and yield (Per plot, $R_s = -0.11$, $P = 0.16$, $n = 180$; Per variety, $R_s = -0.05$, $P = 0.75$, $n = 45$). Ear emergence and leaf senescence were recorded for all plots to investigate possible relationships between crop development, earliness phenotypes and TAB. This could not be suitably explored due to the restricted inoculum build-up over the whole trial site. However, analysis of data did detect a very weak positive correlation between TAB and leaf senescence recorded on the 20th July per plot ($R_s = 0.18$, $P = 0.01$, $n = 180$), although this was not significant when analysing variety means ($R_s = 0.01$, $P = 0.95$, $n = 45$).

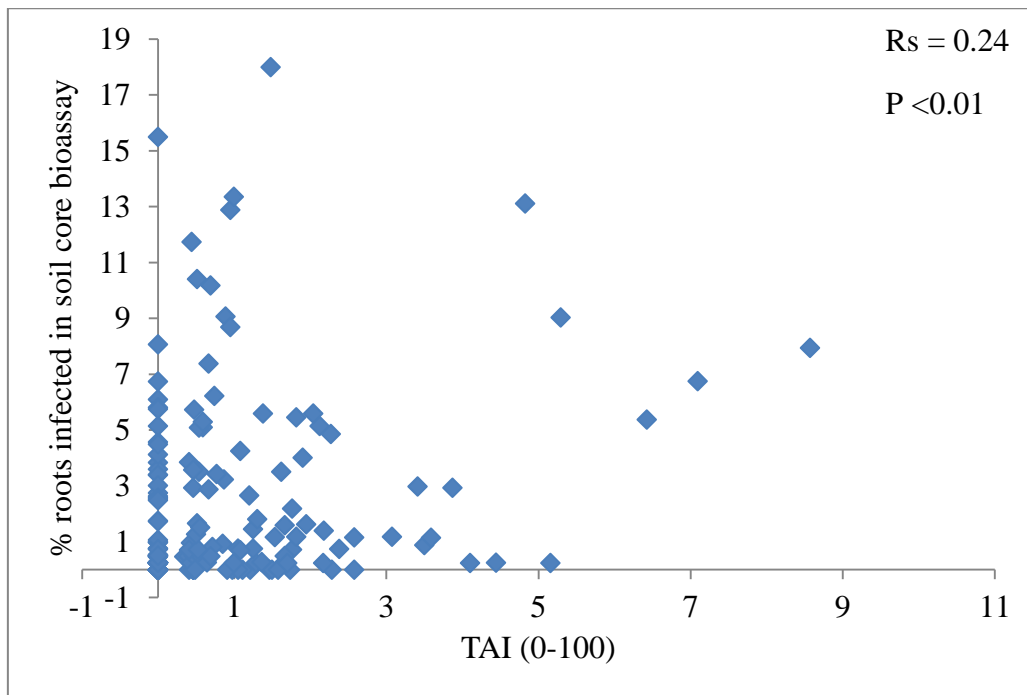
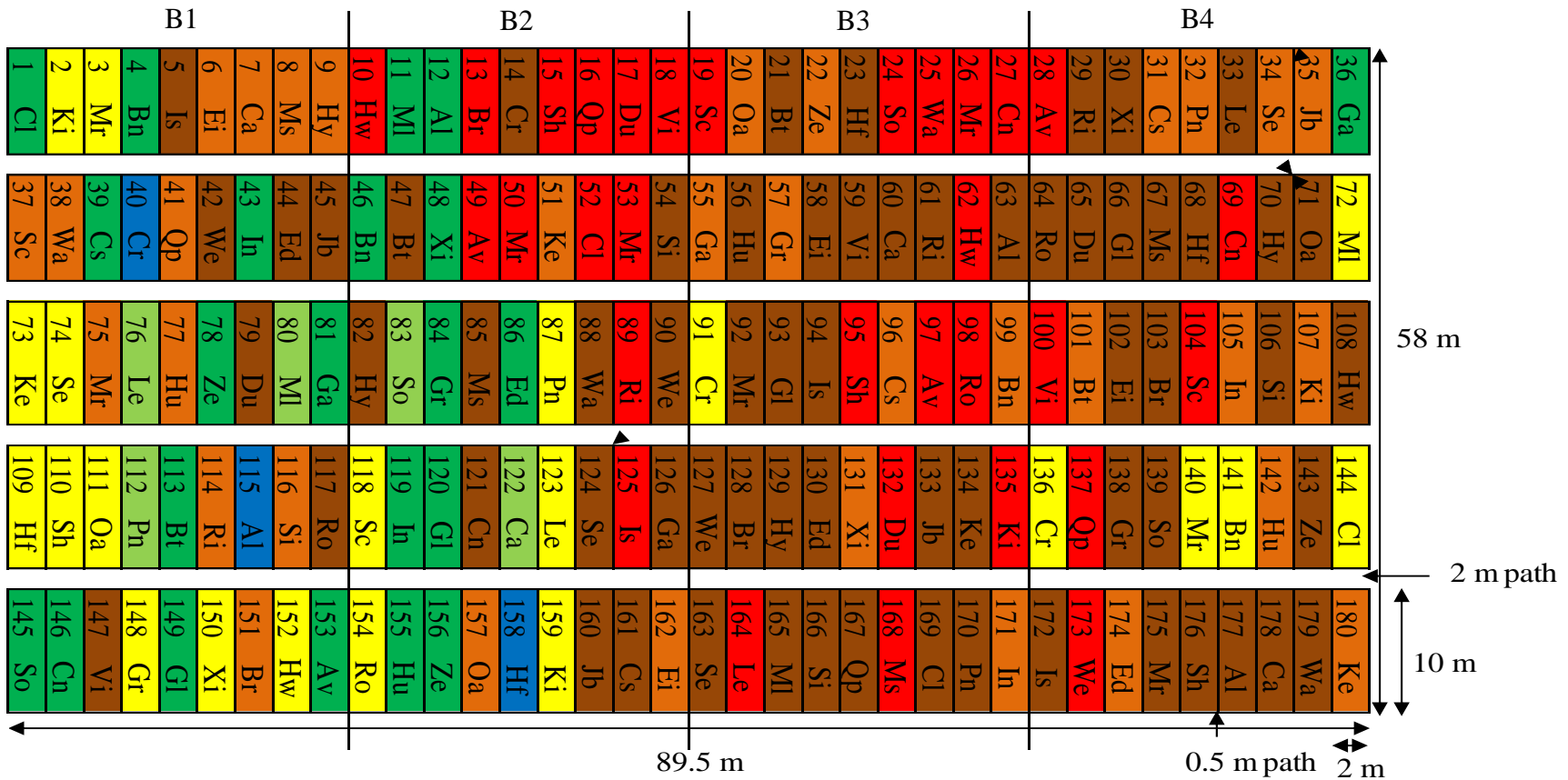


Figure 3.3. Per plot correlation between the percentage roots infected on bait plants in the soil core bioassay and the take-all index of plant samples in the 2010 first wheat field trial (10/R/WW/1032).

In 2011 levels of take-all inoculum over the field trial (11/R/WW/1115) ranged hugely from 6 to 69% roots infected on bait plants in the soil core bioassay (back transformed varietal means; Table 3.7). The per plot percentage roots infected in the soil core bioassay mapped onto the field plan show the unusual distribution of take-all inoculum over the 2011 trial site, compared with the distribution in the 2009 trial (Figures 3.4 and 3.5). When the soil core bioassay plants in 2011 were assessed the fungus *Phialophora graminicola* was identified at moderate levels, typically associated with bioassay plants showing a lack of black take-all lesions. *P. graminicola* is weakly parasitic and has been shown to delay take-all epidemics from developing (Slope et al., 1978, Slope et al., 1979). In addition the plant samples assessed from the 2011 trial showed moderate levels of disease in some parts of the trial (Table 3.8; TAI variety mean range: 1.70 to 26.51). Take-all disease on the roots is usually negligible in first wheat crops (Hornby et al., 1998) so this probably indicates that in some areas of the field there was a carry-over of take-all inoculum through the break crop before sowing the first wheat 2011 trial. The TAI of plant samples was significantly positively correlated with TAB at both the plot and variety levels (Per plot, $R_s = 0.70$, $P < 0.001$, $n = 180$, Figure 3.6; Per variety, $R_s = 0.50$, $P < 0.001$, $n = 45$). The presence of *P. graminicola* across parts of the

trial and the carry-over of take-all inoculum in other areas probably explain the highly uneven background variation over the trial as shown in Figure 3.5.

All the correlations between TAB, yields and leaf senescence from the 2011 trial should be treated with caution due to the influence of *P. graminicola* and take-all inoculum carry-over. There was a small yield effect detected with low TAB associated with higher yields (Per plot, $R_s = -0.48$, $P < 0.001$, $n = 180$, Figure 3.7; Per variety, $R_s = -0.34$, $P = 0.02$, $n = 45$). These correlations do not indicate causation; the correlation is probably influenced by the higher than expected levels of take-all root infection of the plant samples in the 1st wheat field. Spearman's rank correlation analysis between TAI (0-100) and yield does also show a significant negative association (Per plot, $R_s = -0.32$, $P < 0.001$, $n = 180$; Per variety, $R_s = -0.37$, $P = 0.01$, $n = 45$). Spearman's rank correlation analysis indicated a slight positive correlation per plot on observations of TAB and leaf senescence recorded on the 11th July ($R_s = 0.24$, $P < 0.001$, $n = 180$) and 20th July ($R_s = 0.28$, $P < 0.001$, $n = 180$).

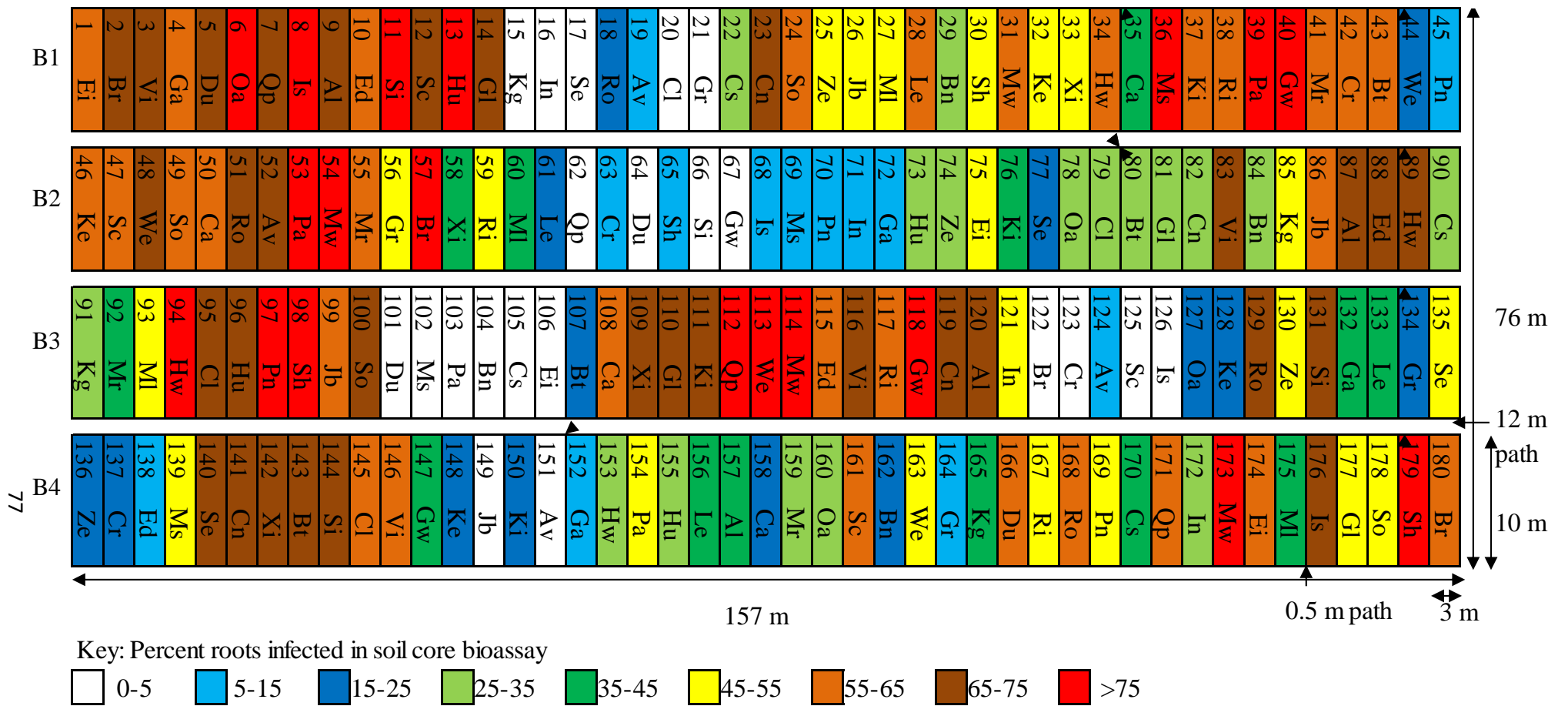


Key: Percent roots infected in soil core bioassay



Figure 3.4. Percentage roots infected in the soil core bioassay per plot in the 2009 elite winter wheat TAB field trial (09/R/WW/916).

Field: New Zealand Treatments: 45 varieties x 4 blocks (B1-B4)



B4
77

B1

B2

B3

76 m
12 m
path
10 m

157 m

0.5 m path
3 m

45	Pn	90	Cs	135	Se	180	Br
44	We	89	Hw	134	Gr	179	Sh
43	Bt	88	Ed	133	Le	178	So
42	Cr	87	Al	132	Ga	177	Gl
41	Mr	86	Jb	131	Si	176	Is
40	Gw	85	Kg	130	Ze	175	Ml
39	Pa	84	Bn	129	Ro	174	Ei
38	Ri	83	Vi	128	Ke	173	Mw
37	Ki	82	Cn	127	Oa	172	In
36	Ms	81	Gl	126	Is	171	Qp
35	Ca	80	Bt	125	Sc	170	Cs
34	Hw	79	Cl	124	Av	169	Pn
33	Xi	78	Oa	123	Cr	168	Ro
32	Ke	77	Se	122	Br	167	Ri
31	Mw	76	Ki	121	In	166	Du
30	Sh	75	Ei	120	Al	165	Kg
29	Bn	74	Ze	119	Cn	164	Gr
28	Le	73	Hu	118	Gw	163	We
27	Ml	72	Ga	117	Ri	162	Bn
26	Jb	71	In	116	Vi	161	Sc
25	Ze	70	Pn	115	Ed	160	Oa
24	So	69	Ms	114	Mw	159	Mr
23	Cn	68	Is	113	We	158	Ca
22	Cs	67	Gw	112	Qp	157	Al
21	Gr	66	Si	111	Ki	156	Le
20	Cl	65	Sh	110	Gl	155	Hu
19	Av	64	Du	109	Xi	154	Pa
18	Ro	63	Cr	108	Ca	153	Hw
17	Se	62	Qp	107	Bt	152	Ga
16	In	61	Le	106	Ei	151	Av
15	Kg	60	Ml	105	Cs	150	Ki
14	Gl	59	Ri	104	Bn	149	Jb
13	Hu	58	Xi	103	Pa	148	Ke
12	Sc	57	Br	102	Ms	147	Gw
11	Si	56	Gr	101	Du	146	Vi
10	Ed	55	Mr	100	So	145	Cl
9	Al	54	Mw	99	Jb	144	Si
8	Is	53	Pa	98	Sh	143	Bt
7	Qp	52	Av	97	Pn	142	Xi
6	Oa	51	Ro	96	Hu	141	Cn
5	Du	50	Ca	95	Cl	140	Se
4	Ga	49	So	94	Hw	139	Ms
3	Vi	48	We	93	Ml	138	Ed
2	Br	47	Sc	92	Mr	137	Cr
1	Ei	46	Ke	91	Kg	136	Ze

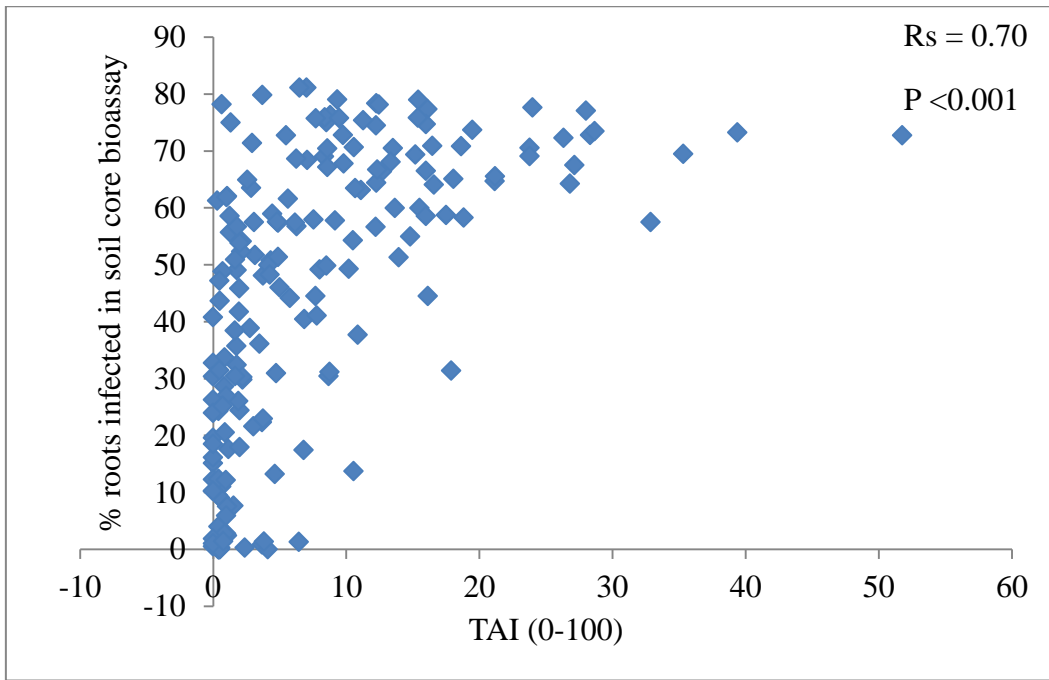


Figure 3.6. Per plot correlation between the percentage roots infected on bait plants in the soil core bioassay and the take-all index of plant samples in the 2011 first wheat field trial (11/R/WW/1115).

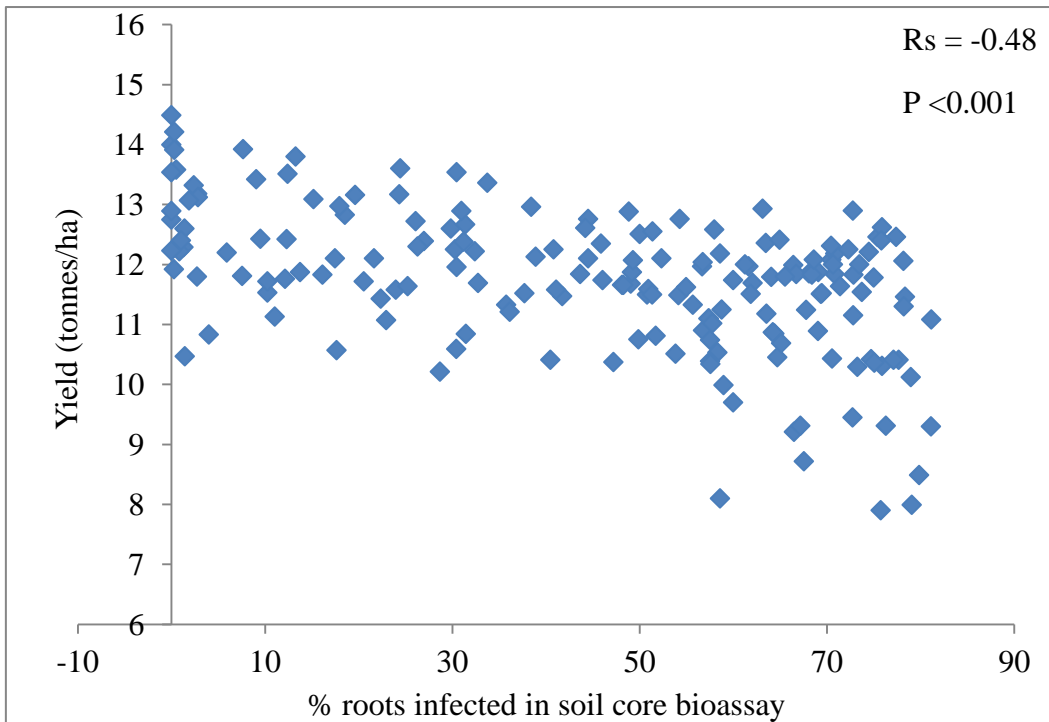


Figure 3.7. Per plot correlation between the percentage roots infected on bait plants in the soil core bioassay and yields in the 2011 first wheat field trial (11/R/WW/1115).

3.3.2. Epidemiology studies 2009-2011

Epidemiology studies show a baseline level of roots infected in the soil core bioassay of around 10% in April of 2009 and 2010 (Tables 3.9b and 3.10b). In 2010 and 2011, when sampling was started a month earlier in March, the soil was already infective, although less than 5% of roots were now infected on bait plants from the soil bioassay (Tables 3.10b and 3.11b). In 2009, the level of take-all inoculum in the soil was not significantly different between April and May, but from May onwards there are significant increases in take-all inoculum at each sampling month, with the greatest increase from July until the after harvest sampling date in August (Table 3.9b). The greatest increase from July to August is probably partly a reflection of the greater length of time in between sampling dates in July and August than other dates. Samples were generally taken in the middle of the month, but were taken a week earlier in July (due to suitable conditions for sampling) and were taken towards the end of August after waiting for harvest of the trial. All six varieties show a similar monthly time course of inoculum build-up in 2009, with no significant interaction effect between variety and month (Table 3.9a). There is no main effect of variety on TAB in the epidemiology study. However a significant effect of variety on TAB was detected in the main study where all 45 varieties were sampled after harvest (Table 3.7). Interestingly in 2009 the two 'high' inoculum builders Avalon and Hereward show a trend towards a 'stall' in inoculum build-up from June to July but then increase rapidly again through to harvest (Table 3.9a). In contrast levels of take-all inoculum in the soil in 2010 generally decline from April to July, probably due to the dry weather (Tables 3.10a and 3.10b). There was no effect of variety on TAB in the epidemiology study or in the main after harvest study of all 45 varieties in 2010.

In 2011, the epidemiology study shows mixed trends; take-all inoculum tends to increase from the beginning of June onwards but the varieties Avalon and Cordiale stay generally low throughout (Table 3.11a). Very low soil moisture levels (less than 0.04 m³ water/m³ soil) were recorded in field at the start of data logging in late May and the beginning of June 2011 (Figure 3.8). Soil moisture then increased from the 6th June to a maximum of 0.233 m³ water/m³ soil on the 25th June, and generally stays above 0.1 m³ water/m³ soil for the rest of the growing season. Maximum soil temperatures range from 13.2°C to 23.8°C. Soil cores for the epidemiology study could not be taken in May as the ground was too hard and dry, so were instead taken on the 07th June and again on the 21st June. From April to the first sampling date in June there is only a slight increase in

inoculum build-up, perhaps reflecting the limiting effect of soil moisture on build-up. The rate of build-up increases from June onwards, when soil moisture levels are higher (Table 3.11b). A main effect of variety on TAB was identified in 2011 (Table 3.11b), and there was also a close to significant interaction between variety and sampling date (Table 3.11a). This significant effect is however unlikely to be due to genuine varietal differences but rather due to the unusual distribution of inoculum in the 2011 trial. By chance, three of the four Avalon (Av) plots are in areas of the trial with low take-all inoculum build-up (Figure 3.5) due to the presence of *Phialophora graminicola*. Avalon has previously been a consistently high TAB variety in five years of WGIN field trials and in the 2009 elite winter wheat TAB trial (2004-2008, McMillan et al. 2011; 2009, Table 3.7). When all plots were sampled after harvest there was a non-significant ($P = 0.465$) difference between all 45 varieties (Table 3.7), reflecting the high level of 'patchiness' in the field trial (Figure 3.5), which was probably not detected in the epidemiology study when only 6 varieties were sampled (Table 3.11b).

Table 3.9a. Epidemiology study on take-all inoculum build-up from April through to harvest under six winter wheat varieties in the 2009 elite winter wheat TAB field trial (09/R/WW/916).

Variety	Logit % roots with take-all (back-transformed mean)				
	Month				
	April	May	June	July	After harvest
Avalon	-1.10 (9.5)	-0.95 (12.5)	-0.33 (33.5)	-0.29 (35.3)	0.47 (71.4)
Cadenza	-0.93 (13.0)	-0.82 (15.7)	-0.50 (26.5)	-0.20 (39.5)	0.11 (55.0)
Cordiale	-1.11 (9.3)	-0.83 (15.4)	-0.58 (23.2)	-0.33 (33.4)	-0.02 (48.4)
Hereward	-0.80 (16.3)	-1.07 (9.9)	-0.40 (30.6)	-0.37 (31.7)	0.50 (72.8)
Riband	-0.83 (15.5)	-0.81 (16.0)	-0.72 (18.5)	-0.21 (39.1)	0.38 (67.6)
Xi19	-0.98 (11.8)	-0.70 (19.4)	-0.37 (32.0)	-0.08 (45.4)	0.11 (54.8)
variety*month					
d.f.	69.35				
SED (logits)	0.20				
F Probability	0.323				

Table 3.9b. Main effect of variety and month on take-all inoculum build-up in the 2009 epidemiology study (09/R/WW/916).

Monthly mean	Logit % roots with take-all (back-transformed mean)	Variety mean	Logit % roots with take-all (back-transformed mean)
April	-0.96 (12.4)	Avalon	-0.44 (28.8)
May	-0.87 (14.6)	Cadenza	-0.47 (27.7)
June	-0.48 (27.1)	Cordiale	-0.58 (23.5)
July	-0.25 (37.3)	Hereward	-0.43 (29.3)
After harvest	0.26 (62.1)	Riband	-0.44 (28.8)
		Xi19	-0.40 (30.3)
d.f.	55.79	d.f.	15
SED (logits)	0.08	SED (logits)	0.08
F Probability	<.001	F Probability	0.342

Table 3.10a. Epidemiology study on take-all inoculum build-up from March through to harvest under six winter wheat varieties in the 2010 elite winter wheat TAB field trial (10/R/WW/1032).

Logit % roots with take-all (back-transformed mean)						
Month						
Variety	March	April	May	June	July	After harvest
Avalon	-1.72 (2.6)	-0.97 (12.1)	-1.66 (3.0)	-1.81 (2.1)	-2.52 (0.1)	-2.21 (0.7)
Cadenza	-1.90 (1.7)	-1.12 (9.1)	-1.51 (4.2)	-1.98 (1.4)	-1.89 (1.7)	-2.87 (0.0)
Cordiale	-2.30 (0.5)	-1.21 (7.6)	-1.50 (4.3)	-2.05 (1.1)	-2.13 (0.9)	-2.60 (0.1)
Hereward	-1.79 (2.2)	-1.04 (10.7)	-1.62 (3.2)	-1.48 (4.4)	-1.99 (1.3)	-2.29 (0.5)
Riband	-1.59 (3.5)	-0.95 (12.6)	-1.41 (5.2)	-1.51 (4.1)	-1.66 (3.0)	-2.53 (0.1)
Xi19	-1.64 (3.1)	-0.92 (13.2)	-1.39 (5.4)	-1.62 (3.2)	-2.14 (0.9)	-2.51 (0.2)
variety*month						
d.f.	68.21					
SED (logits)	0.37					
F Probability	0.842					

Table 3.10b. Main effect of variety and month on take-all inoculum build-up in the 2010 epidemiology study (10/R/WW/1032).

Monthly mean	Logit % roots with take-all (back-transformed mean)	Variety mean	Logit % roots with take-all (back-transformed mean)
March	-1.82 (2.0)	Avalon	-1.81 (2.1)
April	-1.03 (10.7)	Cadenza	-1.88 (1.8)
May	-1.51 (4.1)	Cordiale	-1.97 (1.4)
June	-1.75 (2.5)	Hereward	-1.70 (2.7)
July	-2.06 (1.1)	Riband	-1.61 (3.4)
After harvest	-2.50 (0.2)	Xi19	-1.70 (2.7)
d.f.	55.04	d.f.	15
SED (logits)	0.14	SED (logits)	0.20
F Probability	<.001	F Probability	0.508

Table 3.11a. Epidemiology study on take-all inoculum build-up from March through to harvest under six winter wheat varieties in the 2011 elite winter wheat TAB field trial (11/R/WW/1115).

Logit % roots with take-all (back-transformed mean)						
Month						
Variety	March	April	June (7 th) ¹	June (21 st)	July	After harvest
Avalon	-1.82 (2.1)	-2.46 (0.2)	-2.08 (1.0)	-2.13 (0.9)	-1.90 (1.7)	-0.92 (13.2)
Cadenza	-1.33 (6.1)	-1.18 (8.1)	-1.01 (11.1)	-0.83 (15.4)	-0.27 (36.2)	-0.17 (41.3)
Cordiale	-1.52 (4.1)	-1.79 (2.2)	-2.06 (1.1)	-1.77 (2.3)	-1.21 (7.6)	-1.20 (7.9)
Hereward	-1.53 (4.0)	-1.34 (5.9)	-0.93 (13.0)	-0.53 (25.3)	-0.06 (46.7)	0.22 (60.3)
Riband	-1.38 (5.5)	-1.25 (7.0)	-0.95 (12.6)	-0.66 (20.7)	-0.26 (36.6)	0.12 (55.7)
Xi19	-1.05 (10.5)	-0.94 (12.7)	-0.48 (27.1)	-0.31 (34.5)	0.02 (50.6)	0.16 (57.2)
variety*month						
d.f.	25.62					
SED (logits)	0.503					
F Probability	0.061					

¹ Ground was too hard and dry to take soil cores in May; Soil cores taken on June 7th after rainfall.

Table 3.11b. Main effect of variety and month on take-all inoculum build-up in the 2011 epidemiology study (11/R/WW/1115).

Monthly mean	Logit % roots with take-all (back-transformed mean)	Variety mean	Logit % roots with take-all (back-transformed mean)
March	-1.44 (4.9)	Avalon	-1.89 (1.8)
April	-1.49 (4.3)	Cadenza	-0.80 (16.3)
June (7 th)	-1.25 (7.1)	Cordiale	-1.59 (3.5)
June (21 st)	-1.04 (10.7)	Hereward	-0.70 (19.5)
July	-0.61 (22.2)	Riband	-0.73 (18.4)
After harvest	-0.30 (35.1)	Xi19	-0.43 (29.1)
d.f.	61.34	d.f.	15
SED (logits)	0.111	SED (logits)	0.437
F Probability	<.001	F Probability	0.028

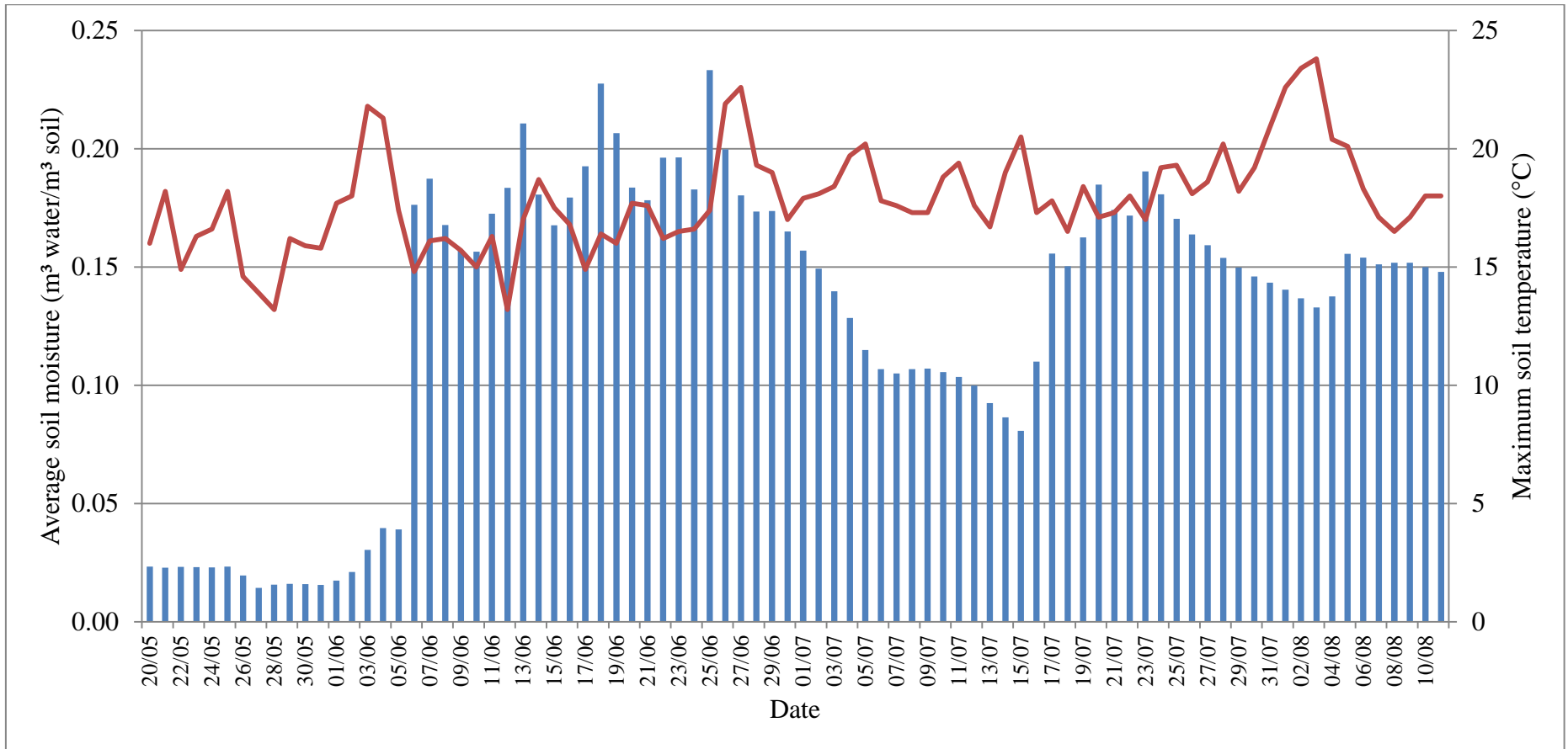


Figure 3.8. Soil moisture (m^3 water/ m^3 soil) (blue bars) and maximum soil temperature ($^{\circ}\text{C}$) (red line) recorded at a depth of 15cm for the 2011 elite winter wheat TAB field experiment 11/R/WW/1115 (Pastures field) from 20th May until August 11th 2011.

Soil samples for pH analysis taken at monthly intervals from the 2011 field trial (11/R/WW/1115) show an increase in soil pH during the growing season with mean pH significantly higher in July and August than in April and June 7th (Table 3.12b). Although there is a trend for a higher soil pH under Hereward no main effect of variety on soil pH was detected ($P < 0.173$; Table 3.12b). No interaction was detected between variety and monthly sampling date (Table 3.12a). Spearman's rank correlation revealed no significant relationships between soil pH and the percentage roots infected in the soil core bioassay per plot at any individual sampling date or when means per plot averaged over all sampling dates were compared (correlation data not shown).

Table 3.12a. Epidemiology study on soil pH under winter wheat varieties Hereward and Cadenza in the 2011 elite winter wheat and take-all inoculum build-up field trial (11/R/WW/1115).

Variety	pH				
	Month				
	April	June (7 th) ¹	June (21 st)	July	After harvest
Cadenza	6.67	6.65	6.74	6.82	6.93
Hereward	6.79	6.83	6.94	6.90	6.96
variety*month					
d.f.	8.89				
SED	0.09				
F Probability	0.433				

¹ Ground was too hard and dry to take soil cores in May. Soil cores taken on June 7th after rainfall.

Table 3.12b. Main effect of variety and month on soil pH in the 2011 elite winter wheat and take-all inoculum build-up field trial (11/R/WW/1115).

Monthly mean	pH	Variety mean	pH
April	6.73	Cadenza	6.76
June (7 th)	6.74	Hereward	6.88
June (21 st)	6.84		
July	6.86		
After harvest	6.95		
d.f.	9.05	d.f.	3
SED	0.05	SED	0.07
F Probability	0.028	F Probability	0.173

3.3.3. Take-all inoculum build-up 2004-2011

The overall mean level of take-all inoculum build-up, averaged over all varieties present in each field trial, varies considerably between years (Table 3.13). The overall level of take-all inoculum in the 2009 field trial was high, with even the lowest building varieties reaching over 42% bait plant roots infected in the soil bioassay (range 42.8%-76.8%, back transformed means). This is likely to be due to the favourable environmental conditions for take-all inoculum build-up during the spring and summer of 2009. The main period of take-all inoculum build-up is from May onwards (Slope & Gutteridge, 1979) and hot, dry weather is known to restrict build-up (Hornby et al., 1998). Over the five years of WGIN first wheat field trials lower temperatures from May-August (below 20°C) and higher rainfall were most conducive to take-all inoculum build-up (McMillan et al., 2011) (Tables 3.13 and 3.14). Although rainfall was low in May 2009, the relatively high rainfall in June and July and moderate temperatures will have encouraged take-all inoculum build-up. The common factor between the highest years of inoculum build-up, 2007 and 2009, appears to be the high level of rainfall in the months of June and July. In terms of crop development in the field this is during anthesis and grain development.

Table 3.13. Mean level of inoculum build-up, measured on bait wheat plants in the soil core bioassay, after harvest of first wheat field trials at Rothamsted Research from 2004 to 2011. Field trials from 2004-2008 were done as part of the Wheat Genetic Improvement Network programme (www.wgin.org.uk). Field trials from 2009-2011 were done as part of my BBSRC-HGCA funded PhD project.

Year	Grand mean: percentage roots infected
2004	18.5
2005	4.4 ¹
2006	19.9
2007	68.2
2008	32.5
2009	61.7
2010	2.2
2011	44.7 ²

¹ In 2005 there was a high incidence of competing *Phialophora* spp. across the whole trial site which restricted the build-up of take-all inoculum

² In 2011 there was a very uneven distribution of take-all across the trial, probably due to the presence of *P. graminicola* restricting TAB in some areas of the trial and the

carry-over of take-all inoculum through the break crop encouraging build-up in other areas.

Table 3.14. Monthly rainfall (mm) and average maximum temperatures (°C) recorded at Rothamsted from March to August for the field seasons from 2004 to 2011 (data from the electronic Rothamsted Archive; e-RA).

Rainfall (mm)							
Year	March	April	May	June	July	August	Total
2004	47	82	52	32	50	113	376
2005	43	66	44	44	39	59	295
2006	50	51	89	15	36	110	351
2007	58	3	136	72	87	64	420
2008	109	54	87	35	90	108	483
2009	37	47	25	68	73	64	314
2010	45	18	39	24	32	128	286
2011	10	5	24	83	45	81	248

Temperature (Average tmax °C)							
Year	March	April	May	June	July	August	Mean
2004	9.6	13.5	16.3	20.3	21.4	22.4	17.3
2005	10.0	13.1	15.8	20.6	20.9	21.3	17.0
2006	7.9	12.5	16.4	21.6	26.1	20.3	17.5
2007	11.1	16.6	16.0	19.2	19.7	20.0	17.1
2008	9.3	12.3	18.0	18.8	20.9	20.1	16.6
2009	11.3	14.7	17.1	19.9	20.8	21.9	17.6
2010	9.8	14.1	15.4	20.8	22.8	19.8	17.1
2011	10.7	17.6	17.4	19	19.9	19.9	17.4

Spearman’s rank correlations of the mean percentage roots infected in the soil core bioassay each year and the monthly rainfall or average maximum temperature in each year were carried out to identify the key environmental conditions and months that were associated with the overall level of take-all inoculum build-up (Table 3.15), excluding 2005 and 2011 due to the presence of *Phialophora* spp. A strong, close to significant, negative relationship was detected between the average maximum temperature in July and mean TAB ($R_s = -0.83$, $P = 0.06$, $n = 6$; Figure 3.9) and a strong positive relationship between rainfall in June and mean TAB ($R_s = 0.83$, $P = 0.06$, $n = 6$; Figure 3.10), indicating that lower temperatures in July and higher rainfall in June are correlated with higher levels of take-all inoculum build-up. Surprisingly, rainfall in August was strongly negatively associated with mean TAB, with lower rainfall correlated with higher mean TAB ($R_s = -0.99$, $P = < 0.01$, $n = 6$; Figure 3.11). This is counter intuitive as dry weather is known to restrict inoculum build-up. This finding is further discussed below in section 3.4.

Table 3.15. Spearman’s rank correlation between the monthly rainfall (mm) and average maximum temperatures (°C) recorded at Rothamsted from March to August and the mean TAB after harvest in the 2004-2011 first wheat field trials (excluding 2005 and 2011 due to *Phialophora* spp.).

Rainfall	Spearman’s rank correlation	
	Rs ¹	P
March	0.31	0.56
April	-0.37	0.50
May	0.37	0.50
June	0.83	0.06
July	0.77	0.10
August	-0.99	<0.01

Temperature	Rs	P
March	0.43	0.42
April	0.43	0.42
May	0.37	0.50
June	-0.66	0.18
July	-0.83	0.06
August	0.03	1.00

¹Rs = Spearman’s rank correlation coefficient.

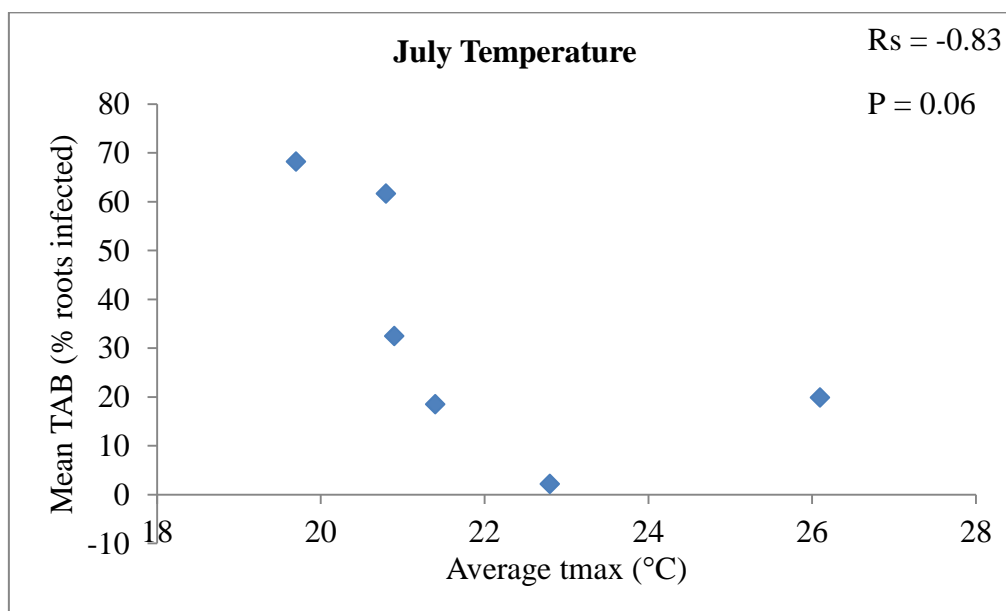


Figure 3.9. Spearman’s rank correlation between average maximum temperature in July and mean TAB in the 2004-2011 first wheat field trials (excluding 2005 and 2011 due to *Phialophora* spp.).

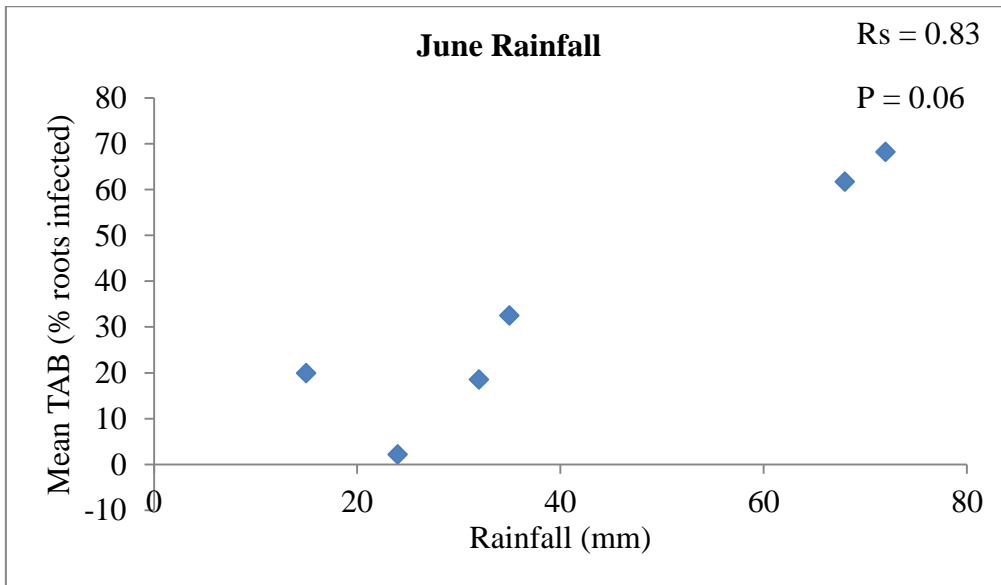


Figure 3.10. Spearman's rank correlation between rainfall in June and mean TAB in the 2004-2011 first wheat field trials (excluding 2005 and 2011 due to *Phialophora* spp.).

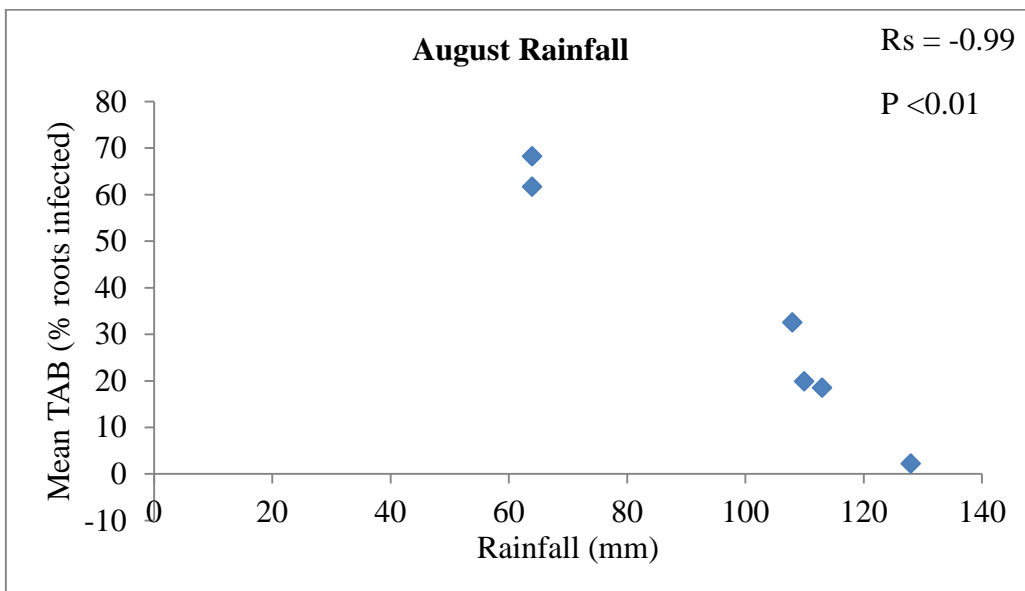


Figure 3.11. Spearman's rank correlation between rainfall in August and mean TAB in the 2004-2011 first wheat field trials (excluding 2005 and 2011 due to *Phialophora* spp.).

3.3.4. Rotation experiments

After harvest in the first year of rotation trial 1 (09-10/R/CS/688) low levels of *Phialophora graminicola* were identified on soil core bioassay plants throughout the trial. The presence of the weakly parasitic and competing population of *P. graminicola* probably reduced the build-up of take-all inoculum in year 1 (mean TAB over trial site = 21.6% roots infected in soil core bioassay; Table 3.16). This is a low mean value

when compared with the 2009 elite winter wheat and TAB trial which had a mean build-up of 61.7% roots infected in the soil core bioassay (09/R/WW/916; section 3.3.1). This trial was conducted in the same year but in a field on the Rothamsted farm without a resident *P. graminicola* population. Despite the lower take-all build-up in year 1 across the rotation trial field there was a significantly higher take-all build-up in the soil from the Hereward plots than the Cadenza plots (Table 3.16). In the spring and summer of year 2 there was a trend for greater take-all incidence and severity in the 2nd wheat plots after Hereward than Cadenza, but this was only significant at the summer sampling point. Yields after Hereward were on average 0.44 tonnes/ha lower than after Cadenza, although this was not significant ($P = 0.190$; Table 3.16).

There was no interaction effect detected between first wheat ‘source’ variety and the second wheat ‘oversow’ variety for any variable analysed. The main effect of year 2 ‘oversow’ variety is shown in Table 3.17. Unexpectedly there was a significant effect of year 2 ‘oversow’ variety on the percentage roots infected in the soil core bioassay after harvest in year 1. This was before the oversow varieties were sown and suggests that the random allocation of variety treatments in year 2 was unfortunate and correlated with the uneven distribution of TAB, so that some variety treatments are on plots with generally higher levels of take-all inoculum and other variety treatments on areas with lower levels of take-all inoculum. To investigate the potential effect of this on mean take-all severity of the ‘oversow’ varieties in the spring and summer of year 2 the percentage roots infected with take-all after harvest in year 1 was included as a covariate in the ANOVA structure. There was no significant effect of the covariate on take-all in the spring or summer for the ‘oversow’ varieties, and adjusted means were not altered very much. The original analysis, without the added covariate, is therefore displayed in Table 3.17. The main effect of ‘oversow’ variety in year 2 revealed no significant differences on take-all incidence and severity in the spring but a significant effect in the summer. The variety Robigus had the most severe take-all and Solstice the lowest level of take-all, demonstrating potential differences in susceptibility to take-all within elite winter wheat varieties. It is hard to compare the effect of take-all on yield for different varieties as there are inherent yield differences between varieties even in the absence of take-all. In this case Robigus was one of the higher yielding varieties in the trial, despite the increased severity of take-all in these plots. This is also unexpected as in general Robigus is known as a ‘bad’ second wheat in terms of yield (see Table 3.5).

Table 3.16. Rotation trial 1 (Year 1: 09/R/CS/688; Year 2: 10/R/CS/688). Take-all infectivity of the soil after the first wheat source varieties Cadenza and Hereward, and take-all disease and yield data in the subsequent second wheat oversow.

Source variety	Year 1	Year 2 ³		Oversow		
	Soil bioassay after harvest of 1 st wheat plots ¹	Oversow Spring plant samples	Take-all roots per plant	Oversow Summer plant samples	Oversow Yields	
	Logit % roots infected (BT ² means)	Logit % plants with take-all (BT means)		Logit % plants with take-all (BT means)	TAI (0-100)	tonnes/ha
Cadenza	-1.01 (11.1)	-1.21 (7.7)	0.20	-0.83 (15.4)	8.3	10.82
Hereward	-0.67 (20.1)	-0.90 (13.9)	0.35	-0.28 (35.8)	18.5	10.38
d.f.	3	3	3	3	3	3
SED	0.08	0.17	0.07	0.15	2.91	0.26
F Probability	0.027	0.158	0.118	0.033	0.040	0.190
Grand mean	-0.84 (15.6)	-1.06 (10.8)	0.27	-0.56 (25.6)	13.4	10.60

¹ Moderate levels of *Phialophora graminicola* on soil core bioassay plants across the trial site.

² BT, back-transformed.

³ Seed spill on plots 48 and 64 during sowing of the second wheat varieties, samples not taken from these plots.

Table 3.17. Rotation trial 1 (Year 1: 09/R/CS/688; Year 2: 10/R/CS/688). Take-all infectivity of the soil after harvest of year 1 plots, analysed by the plot locations of the 2nd wheat oversow varieties. Take-all disease and yield data in the subsequent second wheat oversow varieties.

	Year 1	Year 2 ⁴		Oversow		
	Soil bioassay after harvest of 1 st wheat plots ¹	Oversow Spring plant samples	Take-all roots per plant	Summer plant samples	Oversow Yields	
Oversown variety	Logit % roots infected (BT ³ means)	Logit % plants with take-all (BT means)		Logit % plants with take-all (BT means)	TAI (0-100)	tonnes/ha
Solstice	-1.02 (10.9)	-1.17 (8.4)	0.21	-0.94 (12.8)	8.0	10.36
Xi19	-0.85 (14.9)	-0.96 (12.5)	0.33	-0.72 (18.5)	7.7	10.60
Einstein	-0.90 (13.8)	-1.12 (9.3)	0.24	-0.39 (31.2)	17.9	10.71
Hereward	-1.33 (6.1)	-1.09 (9.8)	0.31	-0.55 (24.4)	15.1	10.08
Cordiale	-0.66 (20.5)	-0.86 (14.8)	0.39	-0.68 (20.1)	9.9	11.17
Robigus	-0.50 (26.3)	-1.13 (9.0)	0.21	-0.12 (43.6)	25.6	10.85
Gallant	-0.34 (33.0)	-0.88 (14.3)	0.30	-0.36 (32.2)	15.2	9.93
Duxford	-1.14 (8.7)	-1.23 (7.4)	0.19	-0.71 (19.1)	7.8	11.06
d.f.	42	40	40	40	40	42
SED	0.31	0.27	0.13	0.17	4.43	0.37
F Probability	0.043 ²	0.818	0.713	<.001	0.002	0.013
Grand mean	-0.84 (15.6)	-1.06 (10.7)	0.27	-0.56 (25.2)	13.4	10.60

¹ Soil core bioassay was taken after harvest of the source first wheat varieties Cadenza and Hereward, before sowing of the 8 oversow varieties. Soil cores were taken from the location of where the year 2 plots would be sown.

² Significant effect detected of year 2 ‘oversow’ variety on the percentage roots infected in the soil core bioassay after harvest in year 1 (P = 0.043). This was before the oversow varieties were sown and suggests that the random allocation of variety treatments in year 2 was unfortunate and correlated with the uneven distribution of TAB. Logit % roots infected in soil core bioassay after year 1 was therefore included as a covariate in ANOVA structure for analysis of year 2 variables to explore the potential confounding effect on ‘oversow’

variety performance in year 2. No significant effect of the covariate was detected so the original ANOVA (without covariate) results are shown in this table.

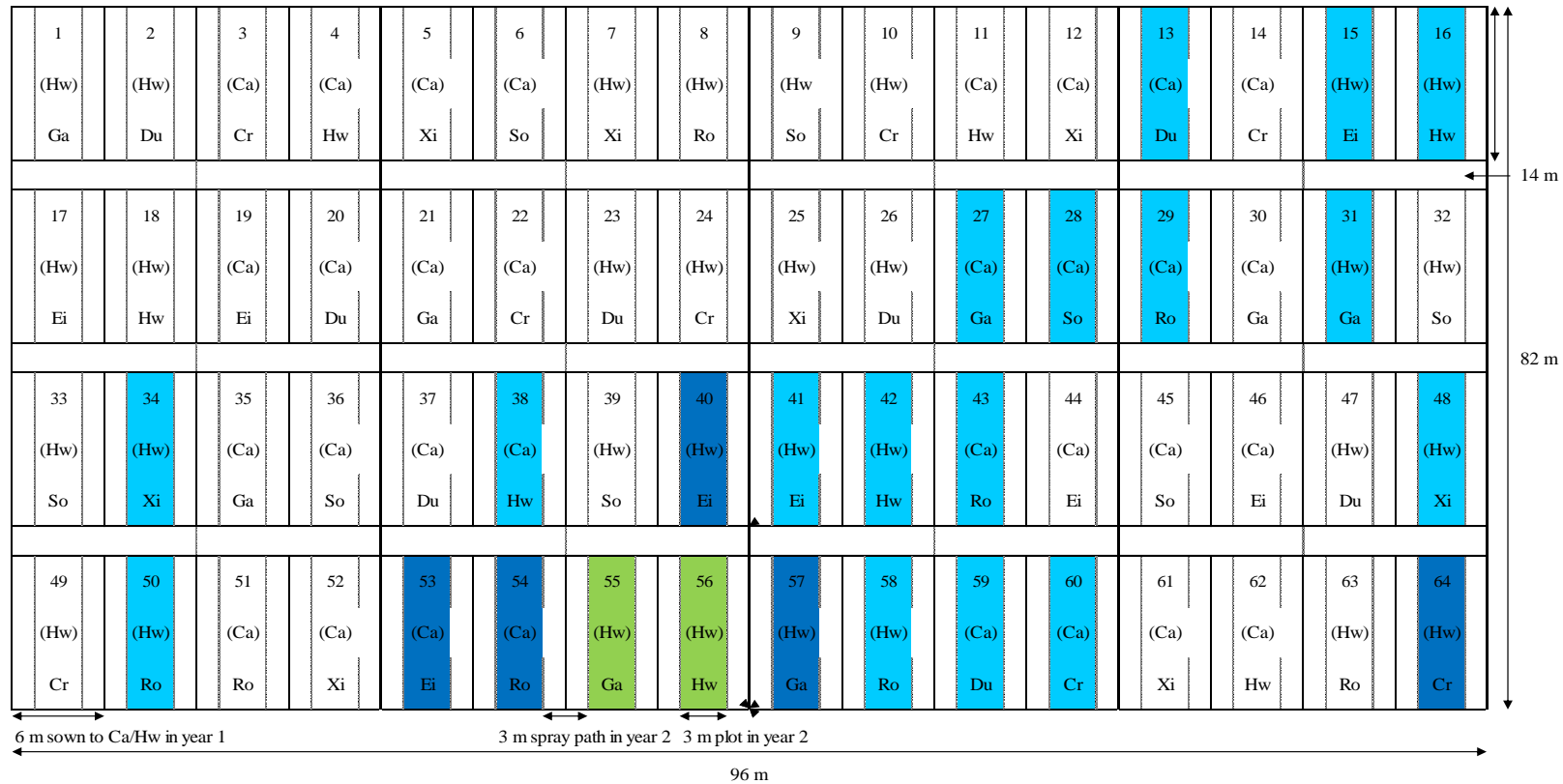
³ BT, back-transformed.

⁴ Seed spill on plots 48 and 64 during sowing of the second wheat varieties, samples not taken from these plots.

In the first year of the second rotation experiment (10/R/CS/706) there was restricted take-all build-up over the majority of the trial site, although there were some small areas around the trial with higher build-up (Figure 3.12), perhaps due to variable soil conditions over the field. The overall low level of build-up (mean: 2.2% roots infected in the soil core bioassay; Table 3.18) was similar to the elite winter wheat and TAB field trial in the same year (10/R/WW/1032; section 3.3.1), and was probably due to the very dry conditions in the spring and early summer of 2010. There was no significant effect of the source variety (Cadenza or Hereward) on TAB measured after harvest in the first year ($P = 0.450$; Table 3.18), perhaps partly due to the patchy pattern of build-up over the trial (Figure 3.12). As should be expected (and in contrast to rotation trial 1, 09/R/CS/688) there was no effect of the after year 1 harvest TAB distribution on the plots randomly allocated for the second year 'oversow' varieties ($P = 0.271$; Table 3.19). In both the spring and summer plant sampling in year 2 there was a trend for significantly higher take-all disease incidence and severity after Hereward as the year 1 'source' variety than after Cadenza (Table 3.18). Yields in the 2nd year were also 0.2 tonnes/ha lower after Hereward than Cadenza ($P = 0.043$). This was unexpected as mean take-all inoculum build-up was extremely low and not significantly different after Cadenza or Hereward in year 1 (1.8% and 2.6% roots infected in the soil bioassay respectively- back transformed means). However, Spearman's rank correlation analysis does show a significant positive association on a per plot basis between the percentage roots with take-all in the soil core bioassay after harvest in year 1 and take-all severity in the summer the following year ($R_s = 0.63$, $P < 0.001$, $n = 64$; Figure 3.13).

There were no significant interactions between year 1 'source' variety and the eight year 2 'oversow' varieties. In year 2 there was no significant effect of 'oversow' variety on take-all disease severity in the spring, but significant effects on take-all disease in the summer were detected (Table 3.19). In the summer the variety Robigus had a significantly higher level of take-all root infection than the other seven year 2 varieties. This is a similar trend as in the first rotation trial. However, the increased severity of take-all on Robigus plants in both the first and second rotation trials may be partly due to the trend for higher build-up after harvest in year 1, by chance, for the plots that were then to be sown with Robigus. In rotation trial 2 there was a mean of 5.9% roots infected in the soil bioassay for plots to be sown with Robigus, compared with mean values of 0.4% to 3.5% for plots to be sown with the other seven year 2 varieties ($P = 0.271$; Table 3.19). This possible influence on take-all severity in year 2 is supported by the significant positive association identified between the mean percentage roots

infected in the post-harvest year 1 soil bioassay and the take-all index per plot and per 'oversow' variety (Figures 3.13 and 3.14). The susceptibility of current elite winter wheat varieties to take-all has been explored in more detail in third wheat field trials presented in Chapter 6.



Key: Percent roots infected in soil core bioassay

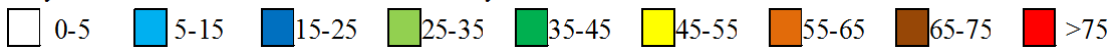


Figure 3.12. Percentage roots infected in the soil core bioassay per plot after harvest of year 1 of Rotation trial 2 (10/R/CS/706).

Field: Great Knott 1 Treatments: Year 1 (in brackets) – Hereward (Hw) and Cadenza (Ca) x 4 reps Year 2 - 8 varieties x 8 reps

Table 3.18. Rotation trial 2 (Year 1: 10/R/CS/706; Year 2: 11/R/CS/706). Take-all infectivity of the soil after the first wheat source varieties Cadenza and Hereward, and take-all disease and yield data in the subsequent second wheat oversow.

Source variety	Year 1	Year 2		Oversow		Oversow
	Soil bioassay after harvest of 1 st wheat plots	Oversow Spring plant samples	Take-all roots per plant	Summer plant samples	TAI (0-100)	Yields
	Logit % roots infected (BT ¹ means)	Logit % plants with take-all (BT means)		Logit % plants with take-all (BT means)		tonnes/ha
Cadenza	-1.87 (1.8)	-1.55 (3.8)	0.05	-0.56 (24.2)	13.49	11.17
Hereward	-1.72 (2.6)	-1.37 (5.5)	0.12	-0.38 (31.5)	21.07	10.97
d.f.	3	3	3	3	3	3
SED	0.17	0.04	0.02	0.10	2.35	0.06
F Probability	0.450	0.021	0.039	0.181	0.048	0.043
Grand mean	-1.79 (2.2)	-1.46 (4.7)	0.08	-0.47 (27.9)	17.28	11.07

¹BT, back-transformed.

Table 3.19. Rotation trial 2 (Year 1: 10/R/CS/706; Year 2: 11/R/CS/706). Take-all infectivity of the soil after harvest of year 1 plots, analysed by the plot locations of the 2nd wheat oversow varieties. Take-all disease and yield data in the subsequent second wheat oversow varieties.

Oversown variety	Year 1	Year 2		Oversow		Oversow
	Soil bioassay after harvest of 1 st wheat plots ¹	Oversow Spring plant samples	Take-all roots per plant	Summer plant samples ³	TAI (0-100)	Yields tonnes/ha
	Logit % roots infected (BT ² means)	Logit % plants with take-all (BT means)		Logit % plants with take-all (BT means)		
Solstice	-1.97 (1.4)	-1.51 (4.1)	0.10	-0.53 (25.2)	12.01	11.19
Xi19	-1.86 (1.9)	-1.36 (5.7)	0.07	-0.59 (23.1)	12.62	12.03
Einstein	-1.69 (2.8)	-1.60 (3.4)	0.07	-0.59 (23.0)	11.97	9.91
Hereward	-1.62 (3.3)	-1.57 (3.7)	0.07	-0.31 (34.3)	20.29	10.54
Cordiale	-1.90 (1.7)	-1.47 (4.5)	0.13	-0.65 (21.1)	15.23	10.30
Robigus	-1.35 (5.9)	-1.44 (4.8)	0.08	0.39 (68.2)	39.89	11.96
Gallant	-1.60 (3.5)	-1.33 (6.0)	0.09	-0.45 (28.4)	17.58	10.07
Duxford	-2.36 (0.4)	-1.40 (5.2)	0.06	-1.01 (11.2)	8.66	12.53
d.f.	42	42	42	42	42	42
SED	0.377	0.283	0.075	0.303	6.941	0.298
F Probability	0.271	0.977	0.977	0.005	0.002	<.001
Grand mean	-1.79 (2.2)	-1.46 (4.7)	0.08	-0.47 (27.9)	17.28	11.07

¹ Soil core bioassay was taken after harvest of the source first wheat varieties Cadenza and Hereward, before sowing of the 8 oversow varieties. Soil cores were taken from the location of where the year 2 plots would be sown.

² BT, back-transformed.

³ One sampling missing (03B).

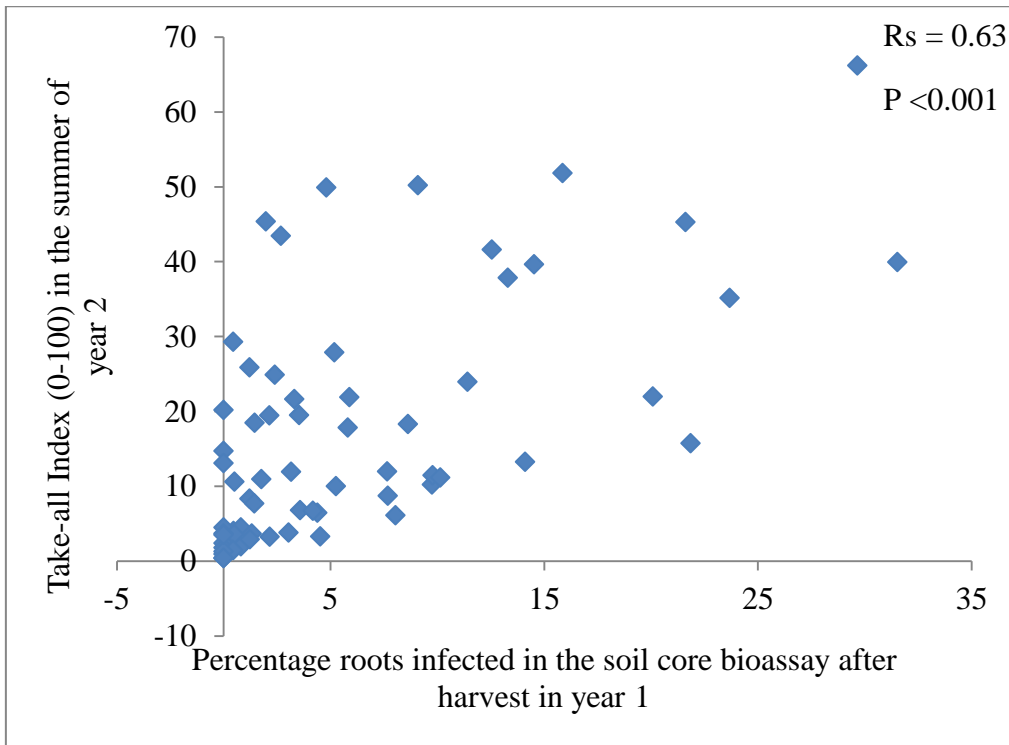


Figure 3.13. Per plot correlation between the percentage roots infected on bait plants in the soil core bioassay after harvest in year 1 of rotation trial 2 and the take-all index of plant samples in year 2 (10-11/R/CS/706).

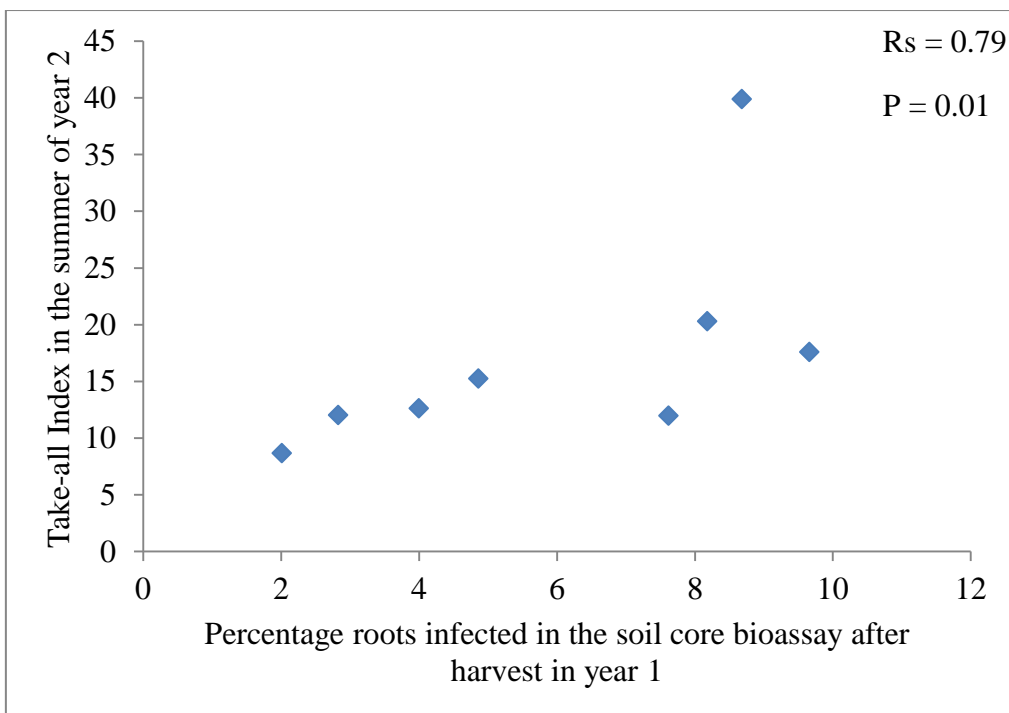


Figure 3.14. Per 'oversow' variety correlation between the percentage roots infected on bait plants in the soil core bioassay after harvest in year 1 of rotation trial 2 and the take-all index of plant samples in year 2 (10-11/R/CS/706).

3.4. Discussion

Three years of field trials were carried out to evaluate the take-all inoculum building ability of current elite UK winter wheat varieties sown as a first wheat crop. The first year (2009) was highly conducive to inoculum build-up and the percentage of roots infected in the soil core bioassay was generally quite high across the whole trial site. In these experiments the TAB trait was evaluated by comparison to varieties with contrasting TAB phenotypes as identified within the WGIN field trials used to study TAB (McMillan et al., 2011). This included the low TAB varieties Cadenza, Cordiale and Xi19, medium builder Riband, and higher TAB varieties, Avalon, Hereward, Malacca and Soissons. In general these varieties performed as expected in the 2009 field trial, with the exception of Malacca which was the lowest take-all inoculum builder in 2009 out of all 45 varieties tested. This highlights the importance of multiple years of field testing to explore TAB and identify varieties with consistent phenotypes. There were 9 previously untested elite wheat varieties with lower take-all inoculum build-up than Cadenza and 5 varieties which built up more inoculum than Hereward in the 2009 trial. However, none of the 9 varieties were significantly different from Cadenza or the 5 varieties from Hereward, suggesting that the amount of take-all build-up within current elite varieties is in the same range as the older varieties studied in the WGIN field trials.

Studying take-all inoculum build-up during the growth of a first wheat crop cannot be accurately reproduced in glasshouse studies (R.J. Gutteridge, unpublished data), so is dependent on the use of time consuming, labour intensive field trials. Take-all is known to be a notoriously 'patchy' disease in the field and is greatly influenced by changes in environmental conditions (Hornby et al., 1998). This makes it a challenge to suitably explore treatment effects. However, field trials repeated over multiple years, trial designs with appropriate blocking, and 3 (WGIN trials 2004-2008) or 4 (PhD trials 2009-2011) replicates of each treatment can help to overcome this. Some of the problems associated with take-all field research are illustrated by the 2010 and 2011 elite winter wheat trials carried out in this PhD study. The aim was to confirm the inoculum building ability of the elite varieties in the 2009 trial. 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.

Slope and Gutteridge (1979) demonstrated that May onwards in a first wheat crop is the key time period during which take-all inoculum increases in a winter wheat crop under UK conditions. In the especially dry years of 1975 and 1976 there was little increase in inoculum during the spring and take-all inoculum in the soil at harvest was similar to at the beginning of season when the wheat crop was sown in the autumn after the break crop (Slope et al., 1977). In the WGIN inoculum build-up study (McMillan et al., 2011) we discussed how the level of inoculum build-up in any individual year is greatly influenced by environmental conditions. In the 2004 and 2006 WGIN trials it was fairly dry in the early summer and take-all inoculum detected after harvest was relatively low, but varietal effects were still detected. However, in 2010 it was exceptionally dry during the spring and summer, with widespread drought symptoms of leaf rolling and premature ripening visible in wheat fields across the Rothamsted farm. As in the study by Slope et al. (1977) the soil epidemiology study in this year showed only a slight increase in take-all inoculum in the soil in the early spring from March to April. There was then a decline in inoculum levels during the rest of the season with nearly undetectable inoculum levels after harvest under all varieties in the trial. In an ideal situation trials would be irrigated during the first wheat crop if the weather was exceptionally dry as in 2010. Unfortunately it is not practical or possible to irrigate the large number of trials that the take-all research group have running in any one year. This is likely to become more of a problem if the number of especially dry springs increases. At the moment a year like 2010 is relatively unusual. If this became more common it may be necessary to locate trials away from the Rothamsted farm in other wetter parts of the country to ensure good conditions for the study of take-all disease.

The simple correlation analysis, carried out by combining the WGIN trials and the three PhD trials (excluding 2005 and 2011 due to *Phialophora* spp.), revealed lower temperatures in July and higher rainfall in June were the key events associated with years of greatest take-all inoculum build-up. This is in agreement with information in Hornby et al. (1998) that moderate temperatures and high rainfall are generally regarded as encouraging take-all development. Conversely in the correlation analysis high rainfall in August was significantly associated with the years of lowest build-up. This is misleading and is probably the result of the pattern of rainfall across months in the years of lowest build-up. The field trials in 2004, 2006, and 2010 had the lowest levels of overall inoculum build-up and are characterised by the lowest rainfall in June and July, but the highest rainfall in August. This probably indicates that the higher rainfall in

August is too late to encourage the build-up of inoculum after such dry weather in the preceding months.

Correlation analysis of temperature and rainfall in individual months can be deceptive when analysing a limited number of years of data. Within field soil moisture and temperature recordings, as in the 2011 field trial, combined with epidemiology studies would help to provide more specific information on the soil conditions which favour inoculum build-up. Data collected from additional years and different sites in the same year is needed to carry out an accurate analysis of the effect of environmental conditions on TAB. A 'Window Pane' analysis has been used by Beest et al. (2008, 2009) to identify critical time periods and environmental conditions that are linked to damaging levels of *Septoria* leaf blotch, powdery mildew and yellow rust. This information could then be potentially used as an early warning system and decision making guide by farmers to select appropriate control measures (primarily chemical sprays) when the risk of these foliar diseases is high. In the case of take-all, if sufficient data could be collected, a 'Window Pane' analysis could provide valuable information on the inoculum build-up and weather relationship.

The presence of high levels of *Phialophora graminicola* and *Phialophora* sp. lobed hyphopodia in the soil in the 2005 WGIN field trial and *P.graminicola* in parts of the 2011 elite winter wheat trial greatly restricted the development of take-all. The build-up of inoculum was also reduced in the first rotation trial (2009-2010) by low levels of *P. graminicola*. Both of these *Phialophora* spp. are anamorphs of *Gaeumannomyces* spp. (Gams, 2000, Freeman & Ward, 2004). They are only weakly pathogenic and are confined to the epidermis and root cortex so are not thought to cause any significant damage to cereal plants under field conditions (Hornby et al., 1998). Early work by Deacon (1973a) and Slope et al. (1979) described the control of take-all due to significant populations of *Phialophora graminicola* which had developed in grass leys over two or more years prior to wheat being sown. Martyniuk & Myśków (1984) and Wong et al. (1996) have reported on the use *P. sp* lobed hyphopodia as a potential biocontrol agent against take-all (see Chapter 1, section 1.6.2, for more information on biological control of take-all by *Phialophora* spp.) 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. In the early 2000s Pastures field on the Rothamsted farm was used successfully for take-all research which suggests the development of *Phialophora graminicola*, which disrupted the 2011 elite winter wheat trial, is more recent. Records of the previous cropping and field experiment history of Pastures field show that a five year mixed crop experiment was carried out from 2004-2008 (2008 field experiment code 08/R/CS/601). This consisted of different wheat, grass mix and grass/clover mix plots. It is possible that the grass and grass/clover mix plots stimulated the development of populations of *P. graminicola* that have persisted in the soil through the following spring barley and oilseed rape crops in 2009 and 2010, before sowing of the 2011 elite winter wheat field trial. The use of different wheat, grass mix or grass/clover mix plots may also explain the irregular distribution of *Phialophora graminicola* across Pastures field. Deacon (1973b) has previously described how *P. graminicola* populations develop under grass and can then be sustained through several successive wheat crops. Historically many of the fields in Rothamsted were sown to grass which may have encouraged the development of *Phialophora graminicola* in some fields. Fosters field, used in the 2005 WGIN trials had both species of *Phialophora*. However, conditions that naturally encourage the development of *P. sp.* lobed hyphopodia are not known (Gutteridge et al., 2006). In Poland Martyniuk (1987) found *P. sp.* lobed hyphopodia under seven species of field grown grass, while *P. graminicola* occurred under all 15 species tested.

The 2011 trial in Pastures field was also unusual because although take-all is usually negligible in first wheat crops the plant samples assessed from the 2011 trial showed moderate levels of disease in some parts of the trial. This was associated with high levels of take-all inoculum build-up and a great deal of background variation across the site. The moderate levels of disease on the roots of the first wheat crop could be due to the carry-over of take-all inoculum by grass weed hosts and barley volunteer plants through the winter oilseed rape break crop prior to sowing of the first wheat trial. Cereal volunteers are generally more common in oilseed rape than other traditional break crops, such as winter beans, used in UK rotations (Hornby et al., 1998). However, this should not usually lead to significant take-all infections in the first wheat crop, but rather allow a greater build-up of inoculum in the first wheat so possibly more severe take-all in a following second wheat crop. The moderate levels of take-all in the 2011 first wheat trial suggest a more serious volunteer or weed infestation than is normal in an oilseed rape break crop. Cereal volunteers and some grass weed species have been shown in a number of studies to maintain or increase inoculum (Dulout et al., 1997,

Monterroso & Juan, 2002, Gutteridge & Hornby, 2003, Monterroso et al., 2004, Bateman et al., 2005, Gutteridge et al., 2006). Dry weather, which inhibits microbial activity, is known to slow down the breakdown of inoculum surviving saprotrophically in the absence of a host crop (Shipton, 1981, Cotterill & Sivasithamparam, 1987a). The very dry weather in 2010 could potentially have helped the carry-over of inoculum through the break crop. The carry-over of inoculum has not previously been a problem in field trials conducted at Rothamsted as break crops are usually very effective at reducing take-all inoculum to negligible levels before the start of a first wheat experiment. To prevent the 2011 situation field testing is now carried out to help select new fields suitable for trials. In the current BBSRC and Technology Strategy Board funded project 'Protecting second wheats through the reduction of low TAB', a joint project with three of the plant breeding companies, pre-field testing has been carried out to ensure good conditions for inoculum build-up. In the early summer during the break crop and before sowing of first wheat crops in the autumn soil samples are taken from 2 or 3 prospective field trial sites and the soil bioassay set up. Bioassay plants are inspected for evidence of *Phialophora* spp. and *Ggt*, to ensure potential sites are free of *Phialophora* populations and that the break crop has effectively reduced take-all inoculum. Sites with carry-over of inoculum or *Phialophora* spp. can then be avoided for trials.

Due to the problems associated with the 2010 and 2011 elite winter wheat trials the performance of the elite varieties in 2009 could not be confirmed. This work was therefore carried forward into a fourth year after the three PhD study trial years. A first wheat trial was set up in October 2011 with 12 winter wheat varieties (Table 3.20). The twelve varieties were chosen primarily based on their performance in the 2009 elite winter wheat TAB trial in the PhD study, and also to give a range of genetically diverse wheats covering the four nabim groups and different wheat breeding programmes. The low TAB variety Cadenza and high TAB variety Hereward were included for comparison. An epidemiology study on four of the twelve varieties (Cadenza, Claire, Hereford and Hereward) is being carried out to detect when differences between varieties occur in the field. In 2009 there were no significant differences between varieties at the monthly sampling dates in the epidemiology study but there was a trend for a larger increase in inoculum from July to August for the high building varieties (Avalon and Hereward) compared with a smaller increase in this final month for the low building varieties (Cadenza and Xi19).

Table 3.20. Winter wheat varieties chosen for 2012 elite winter wheat and take-all inoculum build-up trial (12/R/WW/1211).

Variety	Nabim group	Breeder	Parentage	2009 TAB group ¹
Alchemy	4	Limagrain	Claire x (Consort x Woodstock)	A
Battalion	2	RAGT	98ST08 x Aardvark	B
Cadenza	2	CPB Twyford	Axona x Tonic	B
Claire	3	Limagrain	Wasp x Flame	B
Duxford	4	Syngenta	Solstice x Scorpion 25	D
Gallant	1	Syngenta	(Malacca x Charger) x Xi19	A
Hereford	4	Syngenta	Solist x Deben	A
Hereward	1	RAGT	Norman 'sib' x Disponent	D
Istabraq	4	Limagrain	Consort x Claire	D
Marksman	2	RAGT	98ST08 x Aardvark	C
Robigus	3	KWS	Z836 x 1366	C
Zebedee	3	Limagrain	Claire x Nelson	A

¹ TAB ability in the 2009 elite winter wheat variety trial (09/R/WW/916). Varieties group into quartiles: A = low TAB, D = high TAB.

The WGIN study 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. Studies by Gutteridge et al. (2008) and Bithell et al. (2009) have previously reported a relationship between the percentage of roots infected in the soil core bioassay after harvest of a first wheat crop and the level of disease in the spring or summer of the following second wheat crop. The discovery of the TAB trait suggests that farmers could limit take-all disease in their second wheats by appropriate choice of a 'low building' first wheat variety. As discussed in Chapter 1 take-all severity in second wheats varies from year to year (Werker & Gilligan, 1990). Severe take-all years are generally associated with seasonal weather patterns (Hornby et al., 1998) and other agronomic management factors (Jenkyn et al., 1998, Spink et al., 2002, Cook, 2003, Gutteridge et al., 2003, Gutteridge & Hornby, 2003). High soil moisture levels have been associated with more severe take-all epidemics (Pillinger et al., 2005). Therefore the expected benefit of growing a low TAB variety as a first wheat crop will partly depend on the weather in the following crop. If conditions are very unfavourable for take-all disease development it would be expected that there would be less benefit of growing a low TAB variety in year 1.

The practical significance of varietal differences in TAB is being investigated in rotational trials within the ongoing WGIN programme. 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. In the second year of both trials there was a trend towards higher take-all incidence and severity in the spring after the high TAB variety Hereward plots, but this was only significant in the second rotation trial. In the summer of both trials the take-all index was significantly higher after the high TAB variety Hereward plots in the first year. Yields following Hereward were also lower, although this was only significant in the second rotation trial. The second rotation trial was interesting because take-all inoculum build-up was very low and not significantly different between Cadenza and Hereward in year 1, due to the dry weather in 2010. However, differences were still detected in year 2 take-all disease levels and yields. This shows that the effect on the second wheat crop is not solely down to the amount of inoculum after harvest in year 1 (although a significant association was still detected on a per plot basis between the percentage roots infected after harvest in year 1 and the take-all index in year 2). Perhaps there is an additional long term mechanism/effect of first wheat variety that works to encourage or suppress take-all disease development in the following second wheat crop. There was no interaction 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. There was, however, evidence that second wheat varieties differ in their susceptibility to take-all disease as a whole, suggesting there could be opportunities to further minimise take-all disease in consecutive wheat crops by growing a less susceptible second wheat variety. Susceptibility of wheat varieties to take-all has been investigated in more detail in third wheat field trials reported in Chapter 6. A third rotation trial is currently in

progress (11-12/R/CS/719). The trial site was free of *Phialophora* spp. and conditions in summer 2011 were wet enough to encourage moderate levels of inoculum build-up. At the end of year 1 in autumn 2011 significant differences were detected after harvest of Cadenza and Hereward in the soil bioassay (Cadenza = 18% roots infected, Hereward = 35% roots infected, $P = 0.034$). Year 2 summer samples and yields are still to be assessed. This will provide information on take-all disease and yields in year 2 after higher levels of inoculum build-up in year 1 than the first two rotation trials.

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. As described in the introduction to this chapter 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 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 (McMillan et al., 2011) 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. In 2009 there was no strong correlation between the ability of the 45 varieties to build-up inoculum and the susceptibility of the same 45 varieties to take-all root infections in a third wheat field trial (third wheat study reported in Chapter 6). This suggests that take-all build-up is not related to susceptibility of wheat varieties to take-all. Bithell et al. (2011a) have shown that take-all inoculum in the soil can be very high after growing rye, despite the fact that rye is relatively resistant to take-all. Nilsson & Smith (1981) also reported that grass species that were the most effective hosts of *Ggt*, encouraging the build-up of inoculum and increased severity of disease in a following crop, were not always the most susceptible to root infection.

The epidemiology studies have started to give more fundamental information on the time course of inoculum build-up. The 2009 study showed that inoculum in the soil can build up rapidly under all the varieties tested and the differences between varieties only appeared after harvest when all varieties were sampled. One possibility is that inoculum build-up is favoured by colonisation of senescent root material towards the end of the season, and that varieties differ in their rate of senescence or amount of root senescent material available. Deacon & Henry (1980) and Kirk (1984) both report that senescing root material is important in the progression of take-all epidemics. In 2010 and 2011 leaf senescence was recorded as an indicator to when varieties naturally begin to senesce in the field. It was not possible to properly test for an association with inoculum build-up as there were not good conditions for inoculum build-up in 2010 or 2011. Crop development measurements in future field trials may help to indicate if the timing of crop maturation has any significant influence on the build-up of inoculum. So far the epidemiology studies reported on inoculum build-up from March till harvest. In future studies it would be useful to measure inoculum build-up throughout the whole season from sowing to harvest to look at whether build-up during the autumn occurs and if this is important at influencing the total amount of inoculum after harvest the following year.

In 2011 the soil pH epidemiology study revealed that there was a trend for a slightly higher soil rhizosphere pH under Hereward than Cadenza, although this was not significant. pH was also not related to the amount of take-all build-up on a per plot basis. It is known that take-all can occur in soils with a pH from 5.5 to 8.5 (Hornby et al. 1998), but it is presumably possible that particular pHs within this range could favour the fungus more than others. Christensen et al. (1987) report that take-all was more severe in soil with a pH of 6.0 than 5.5. Activity of other competitive or antagonistic microflora could also be influenced by soil pH (Duffy et al., 1997, Ownley et al., 2003) and chemical soil properties can be important in the development of suppressive soils against take-all and other plant diseases (Hoper & Alabouvette, 1996, Mazzola, 2002). Smiley (1974) reports that soil rhizosphere pH is influenced by wheat variety. Further studies on rhizosphere soil pH during take-all inoculum build-up will confirm whether this could have an effect on build-up under different varieties.

It is not clear exactly where the take-all fungus is physically located in the building up phase within the 1st wheat crop. The bulk soil, the root surface and the rhizosphere in between are the three possible options. Observations that soil infectivity is greater

within cereal plant rows than between rows (Hornby, 1969, Hornby & Henden, 1986, Cotterill & Sivasithamparam, 1987b) suggest that inoculum is located in close association with plant roots and the rhizosphere region. While Grose et al. (1984) have shown that hyphal growth of *Ggt* through the bulk soil in the absence of a living host can occur. However, in the absence of a living susceptible host plant it is well documented that take-all is located primarily in the crop debris of previously infected plants and survives saprotrophically during this time (Hornby, 1981). Perhaps the growth of wheat roots and release of root exudates in the first wheat crop stimulates the growth of take-all inoculum within the crop debris, allowing the take-all fungus to outcompete the other crop debris colonising microbes and so 'build-up'. Alternatively the take-all fungus may be able to grow from the plant debris out into the bulk soil due to changes in soil properties influenced by the first wheat growth. Seemingly some threshold of inoculum has to be reached before root infection can occur. This is not simply a certain 'amount' of take-all inoculum as even in exceptionally high build-up years there is very little root infection of the first wheat crop. Possibly, the overall maturity of the wheat plants in the spring, and / or the harsh winter conditions have enhanced the wheat plants basal resistance to the *Ggt* fungus which restricts root tissue infection at what are quite low soil temperatures. When a second susceptible wheat crop is grown there is usually an initial phase of primary root infection in the autumn from mycelium thought to be surviving in crop debris (Brown & Hornby, 1971). If weather conditions are favourable the following spring this can lead to secondary infections as root to root contact allows the fungus to spread throughout the root system (Hornby, 1981). Possibly, these initial *Ggt* root infections modulate basal defences locally, and so in the spring as temperatures rise the fungus can colonise the roots, root surfaces and surrounding rhizosphere from these initial foci of infection relatively unimpeded. Alternatively, the microbial populations present below 1st and 2nd wheat crops may be highly dissimilar and this may influence the extent of *Ggt* infection.

The presence of the low TAB trait in a range of current elite wheat varieties reported in this study is surprising as the trait was previously unknown to wheat breeders so has not been specially selected for or against in any of the wheat breeding programmes. It is possible that the low TAB trait has been maintained in elite wheats by linkage to other traits of interest. For the low TAB trait to be useful to plant breeders it is important to establish that low TAB is not associated with a negative effect such as lower yields or earliness. So far evidence suggests no obvious negative associations. In fact low TAB varieties had generally slightly higher yields in the first wheat 2009 field trial in the

PhD study. The genetic basis of the low TAB trait is now being investigated within the continuing WGIN programme and this is discussed in Chapter 4. One major problem is how to select for the low TAB phenotype in breeders programmes. On a practical scale the soil bioassay is too time consuming and labour intensive for screening many hundreds of wheat genotypes such as in a commercial breeding programme. The use of the DNA-based molecular method to detect *Ggt* DNA developed in Australia would be quicker but still very time consuming collecting multiple soil samples for testing. An alternative strategy would be to oversow the different first wheat genotype plots with a single wheat variety and record take-all patch scores and yields in year 2. This method is currently being used experimentally (in conjunction with the soil bioassay after year 1) for the current BBSRC and Technology Strategy Board funded project 'Protecting second wheats through the reduction of low TAB'. Disadvantages of this method are that it would take two years before any information was available and environmental conditions in the second year could potentially mask the effect of wheat genotype.

In summary this study has demonstrated that the low TAB trait is present in a range of elite wheat varieties, of different genetic backgrounds. Rotation trials have demonstrated the potential benefit of the low TAB phenotype in a first wheat on take-all severity and yields in a second wheat crop. If the low TAB trait in the elite wheats proves consistent, over sites and seasons, it 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 completely negate 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.

CHAPTER 4: GENETIC EVALUATION OF THE TAB TRAIT

4.1. Introduction

Take-all is one of the most difficult diseases to combat and currently there is no form of genetic control available to farmers. The **take-all inoculum build-up (TAB)** trait is the first possible genetic solution to the take-all problem. The wheat varieties tested within the WGIN study were diverse, including modern and semi-modern wheats from the UK and Western Europe. In McMillan et al. (2011) we discussed how several of the low TAB varieties in the study had unrelated pedigrees suggesting that there was potentially more than one genetic source of the low TAB trait. Within the main PhD study the presence of the low TAB trait was also demonstrated within a range of other current UK National and Recommended List wheat varieties (Chapter 3). Thus an analysis of variety pedigrees was carried out to identify the filial relationships of the varieties tested and common sources of the low TAB trait.

Defining the genetic basis of inoculum build-up is difficult. Many genes, each with a small overall effect, could influence the TAB trait and unique genetic sources may contribute to low take-all inoculum build-up in different wheat varieties. There is no obvious selection pressure for the low TAB trait and it has also not been specially selected for or against in any of the UK commercial wheat breeding programmes. Finding major quantitative trait loci (QTLs) for a complex trait like take-all inoculum build-up (which also may have multiple environmental interactions) is therefore quite challenging. For the low TAB trait to exist in current elite wheat varieties suggests that the trait at least has no major detrimental effect in terms of growth and yield. It is possible that the maintenance of the low TAB genotype is due to close linkage with other desirable trait(s) that have been selected for in the various UK breeding programmes.

As discussed in chapter 3 the field trials needed to generate phenotypic data to be used to map the TAB trait also have their difficulties; they are labour intensive, require careful site selection, are time consuming to complete and vulnerable to environmental variation. Field trials are however, at the moment, the only way by which to measure take-all inoculum build-up. Previous work by my supervisor Richard Gutteridge has shown that take-all inoculum build-up cannot be replicated in laboratory or growth room tests.

Despite these difficulties two major quantitative trait loci (QTLs) were identified by Richard Gutteridge and Kostya Kanyuka in an Avalon (A) x Cadenza (C) doubled haploid (DH) mapping population (see WGIN newsletters October 2009 and November 2010, <http://www.wgin.org.uk/stakeholders/newsletters.php>; Figure 4.1) within the ongoing WGIN programme. This was based on above ground symptoms of take-all (patch score) and yields in an Oakley oversow in year 2 after 62 A x C lines in year 1. Both QTLs for the low TAB phenotype were from the Cadenza parent. Together these two QTLs accounted for 70% of the phenotypic variation in this trait. This makes these two major effect QTLs attractive prospects for further analysis and in the future good candidates for use in wheat breeding programmes. These QTL positions have been confirmed in 2012 using 16 selected lines from the A x C population (BBSRC-TSB, LowTAB project). Further confirmation of these QTLs is also being carried out in field trials with the full set of 204 A x C doubled haploid lines in the WGIN programme.

The location of the two QTLs has not yet been published so in this chapter the chromosomes are designated A and B and the marker loci XM001 to XM009. Within the PhD study the QTL information was initially used to select two Single Sequence Repeat (SSR) markers that map to the two predicted QTL regions. Frequency of Avalon (high TAB) and Cadenza (low TAB) alleles of these two SSR markers was analysed in the other wheat varieties used in the PhD study. The two markers chosen, Marker M004 (chromosome A) and Marker M008 (chromosome B) (Figure 4.2), were predicted to be most closely associated with the TAB trait in the single marker QTL analysis carried out by Kostya Kanyuka. Following this initial analysis, an additional seven SSR markers were selected from the two chromosomes of interest. This was to examine the genetic similarity of the elite wheat varieties to the low and high TAB varieties, Cadenza and Avalon, within the two chromosomes and QTL regions. Three additional markers M001, M002 and M003 were selected for the analysis of chromosome A and markers M005, M006, M007 and M009 were selected for the analysis of chromosome B (Figure 4.2). The presence of Cadenza and Avalon alleles at the marker loci was then compared with the field performance of the wheat varieties in the 2009 elite winter wheat and take-all inoculum build-up trial (09/R/WW/916; Chapter 3). Analysis of the allelic variation for microsatellite loci at QTL regions provides a way to assess the genetic diversity between varieties and can help to detect other novel genetic sources of the trait of interest.

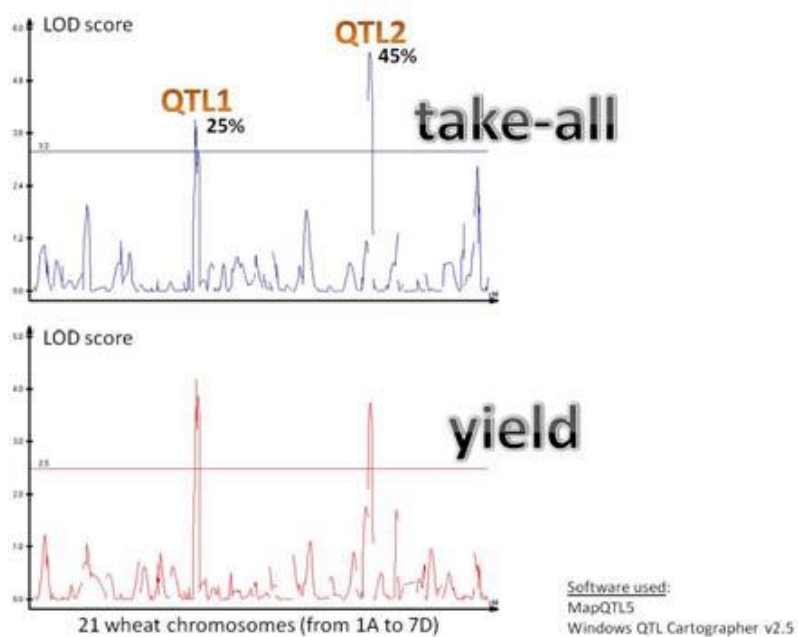


Figure 4.1. Figure reproduced from the WGIN November 2010 newsletter: QTL analysis of field data from the Double Haploid (DH) lines oversown with Oakley.

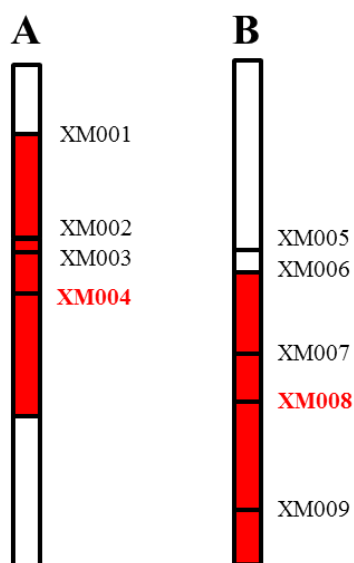


Figure 4.2. Genetic maps of chromosomes A and B showing the location (in red) of the mapped QTLs for low take-all inoculum build-up (TAB). Marker loci in bold and red indicate the loci on each chromosome most closely associated with low TAB trait in the QTL analysis. On the left hand side the chromosome position of loci is shown in centimorgans (cM).

In other studies genetic markers mapped close to QTL have been used to screen germplasm for a particular trait, detect the presence or absence of known QTL, and so

discern the genetic novelty of material exhibiting the trait of interest (Barabaschi et al., 2007, Wang et al., 2007, Sedlacek & Marik, 2010). For example, Gosman et al. (2007) used microsatellite markers linked to known Fusarium Head Blight (FHB) resistance QTLs to investigate the origins of resistance in UK winter wheat varieties. They identified two varieties with potentially novel genetic sources of FHB resistance in UK National List winter wheat varieties. These varieties did not have the same SSR haplotypes as known resistance sources at any of the QTLs previously associated with FHB resistance. In the case of disease resistance this molecular approach allows breeders to select different genetic sources of resistance to help provide more durable disease control or allow pyramiding of resistance genes.

4.2. Materials and Methods

4.2.1. Pedigree analysis

The take-all inoculum building ability of the 45 current wheat varieties screened in field trials within the PhD (Chapter 3) and also those varieties screened in 5 years of WGIN first wheat field trials (McMillan et al., 2011) was used to develop pedigree maps, tracing the possible genetic sources of low and high inoculum build-up. Particular attention was directed at tracing the pedigree of the varieties Cadenza and Avalon, the parent varieties of the doubled haploid mapping population used to identify the genetic basis of the TAB trait within the WGIN programme. The variety Cadenza had been commercially released in 1994 by the breeder CPB Twyford (now KWS UK Ltd., Thriplow, UK), whereas Avalon had been released in 1980 by the breeder PBI (Plant Breeding Institute, Cambridge, UK). An effort was made to identify common sources of low and high TAB trait in the current elite winter wheat varieties.

Wheat pedigree information was obtained from a variety of sources. These included NIAB (National Institute of Agricultural Botany, Cambridge, UK) cereal variety handbooks (yearly publications), old HGCA recommended lists (pedigree information is not included in the more recent lists), the John Innes Centre (Norwich, UK) Cereals Collection website (http://www.jic.ac.uk/germplas/bbsrc_ce/index.htm), a Czech wheat pedigree database with good historic pedigree information provided by the N. I. Vavilov Research Institute of Plant Industry (St. Petersburg, Russia) (<http://genbank.vurv.cz/wheat/pedigree/>), the Scottish wheat variety database containing a comprehensive collection of wheat variety descriptions collated and presented by Science and Advice for Scottish Agriculture (Edinburgh, Scotland)

(<http://wheat.agricrops.org/menu.php>), and also directly from the wheat breeders for some of the most recently released wheat varieties.

4.2.2. SSR analyses

DNA extraction

Ten seeds of each of the 45 elite wheat varieties from the 2009 first wheat field trial (09/R/WW/916) and two additional varieties, Limerick and Paragon (Appendix 4.1), were pre-germinated at room temperature on damp tissue paper for 2 days. After pre-germination three of the germinated seeds of each variety were placed into a well in a p40 seed tray insert filled with Rothamsted standard compost mix (75% medium grade peat, 12% screened sterilised loam, 3% medium grade vermiculite and 10% grit; Petersfield Products, UK), and grown at 20°C in standard Rothamsted glasshouse space. After ten days the seedlings (at the two leaf stage) were harvested for leaf DNA extraction. For each variety approximately 5 cm of leaf tissue was torn off from the second leaf and folded up so that it fitted into the well of a 96 deep well plate. Another 5 cm of leaf tissue was torn off and placed in a second 96 deep well plate. Both 96 deep well plates were stored at -20°C, one for freeze drying and DNA extraction and the second as a spare plate.

DNA was extracted using a laboratory protocol developed at Rothamsted specifically for cereal leaves by Kostya Kanyuka. Leaf tissue was freeze-dried overnight before DNA extraction. Firstly two 3 mm ball bearings were added into each well and the leaf material ground in a TissueLyser (Qiagen Tissue Lyser, Retsch, Germany) for 2 x 2.5 minutes at a frequency of 25-30/sec. Nucleic acids were extracted with the addition of 600 µl DNA extraction buffer (Appendix 4.2) per well and incubation at 65°C for 1 hour on a shaker (Titramax 1000, Heidolph Instruments GmbH & Co., Germany). Following incubation the plates were spun for 10 seconds at 1000 rpm (4-15C, Sigma, UK). Then 200 µl of a 5M solution of potassium acetate (KOAc) (Appendix 4.2) was added per well and plates inverted to mix. The plates were then centrifuged for 30 minutes at 3000 rpm before 500 µl of the supernatant was transferred into chilled *iso*-propanol blocks (prepared by pipetting 275 µl *iso*-propanol per well into a new deep well plate and chilling at -20°C). The *iso*-propanol blocks with supernatant were inverted to mix and incubated at -20°C for 10 minutes. The blocks were centrifuged again for 30 minutes at 3000 rpm to pellet the DNA before the supernatant was poured off and the pelleted DNA was washed with 70% ethanol. After further centrifugation for

10 minutes at 3000 rpm the ethanol was discarded and the nucleic acid pellets were dried at 37°C for 30 minutes. After drying, each pellet was resuspended in 300 µl of sterile distilled water and left overnight at 4°C in the fridge. The next morning the plates were centrifuged for 20 minutes at 3000 rpm to spin down any un-dissolved material. Then 150 µl of the supernatant was transferred into two ordinary 96 v-bottomed well microtiter plates. The amount of DNA extracted was quantified using a NanoDrop machine (NanoDrop 2000C Spectrophotometer, Thermo Scientific, USA). This analysis also assesses the purity of the DNA samples. The entire plant DNA containing plate was then stored at -20°C until future use.

Simple Sequence Repeat (SSR) analyses with infra-red dye (IRD) labeled primers

Nine SSR markers were selected for the study of genetic similarity of current wheat varieties across chromosome locations of interest which contain the putative QTLs conferring low TAB. The 5'-end of the forward primer for each marker was labeled with fluorescent infra-red Li-Cor dye for visualisation (IRD700 or IRD800, LICOR Biosciences, UK). PCR amplifications were carried out in 10 µl reaction mixtures containing 5 µl of 2x PCR Master Mix (Promega), 1 pmol/µl of each primer, 1 µl sterile distilled water and 2 µl of DNA solution (~ 10 ng/µl). Amplifications were carried out in 96-well microtiter plates using a Thermal Cycler (GS4, G-Storm, UK). The PCR thermocycling programme was 5 min at 95°C, followed by 45 cycles of: 30 sec at 95°C, 30 sec at 50°C or 60°C (depending on annealing temperature for individual SSR primers), 1 min at 72°C and a final extension for 5 min at 72°C. Following amplification the PCR products were stored at -20°C.

PCR products were visualised on SequaGel XR® acrylamide gels (EC-842, National Diagnostics, Inc., UK) using a Li-Cor 4300 DNA Analyser (LICOR Biosciences, USA; Appendix 4.3). Gels were pre-run in the Li-Cor DNA Analyser for 25 minutes before loading of the PCR products. The PCR products were diluted 5-30 times using formamide Li-Cor loading dye, denatured for 2 minutes at 95°C, stored on ice before use and then 0.5 µl of the reaction mix was loaded into the wells of the pre-run gel. Electrophoresis was carried out at 1500 V for 90 minutes.

Initially PCR amplification with each marker was carried out using DNA samples from eight doubled haploid lines from the Avalon x Cadenza mapping population developed at the John Innes Centre, Norwich, UK. DNA extractions had already been carried out by Kostya Kanyuka. The lines to test for each marker were selected based on mapping

scores for the Avalon x Cadenza mapping population on the WGIN website (<http://www.wgin.org.uk/resources/MappingPopulation/TAmapping.php>). This was to visualise the marker alleles for the Cadenza (low TAB) and Avalon (high TAB) parents at the marker loci of interest. The genotypes of the other screened winter wheat varieties were then scored by comparing banding patterns to those obtained for Cadenza and Avalon on the basis of the same or different sized PCR products visualised using the Li-Cor gel system.

4.3. Results

4.3.1. Sources of the TAB trait in elite winter wheat varieties: pedigree analysis

The pedigrees of the 45 elite wheat varieties in the 2009 PhD field trial (Chapter 3) are shown in Table 4.1. The parentage of Limerick and Paragon, part of the SSR marker analysis, is also included. The inoculum building abilities of the varieties in the 2009 PhD field trial are also shown in Table 4.1. Varieties have been split into quartile groups (A-D) based on the percentage of bait plant roots infected in the soil core bioassay used to measure inoculum build-up in the field (A = low builders, D = high builders, Trial 09/R/WW/916, see Chapter 3). This information was used to start to construct pedigree maps for varieties of interest, described below.

Table 4.1. Pedigree information of varieties in the 2009 PhD field trial (09/R/WW/916).

Variety	Pedigree	TAB group ²
Alchemy	Claire x (Consort x Woodstock)	A
Avalon	TJB 30/148 x TL 365A/34	D
Bantam	Xi19 x NSL WW35	A
Battalion	98ST08 x Aardvark	B
Brompton	CWW 92.1 x Caxton	D
Cadenza	Axona x Tonic	B
Cassius	Claire x (NSL WW24 x Wizard)	B
Claire	Wasp x Flame	B
Conqueror	Robigus x Equinox	C
Cordiale	(Reaper x Cadenza) x Malacca	A
Duxford	Solstice x Scorpion 25	D
Edmunds	Deben x Napier	B
Einstein	(NHC49 x UK Yield Bulk) x (Haven x Clarion)	C
Gallant	(Malacca x Charger) x Xi19	A
Gladiator	Falstaff x Shannon	B
Grafton	Cordiale x CPBT W97	A
Hereford	Solist x Deben	A
Hereward	Norman sib x Disponent	D

Table 4.1. Continued

Variety	Pedigree	TAB group ²
Humber	Anglo x Krakatoa	B
Hyperion	Aardvark x (Consort x Woodstock)	C
Invicta	NSLWW48 x Robigus	A
Istabraq	Consort x Claire	D
JB Diego	3351b x Stru2374	D
Ketchum	Solstice x Xi19	B
Kipling	Hunter x 9205-4	C
Lear	Robigus x Nijinsky	B
Limerick ¹	Solstice x Scorpion 25	ND
Malacca	Riband x (Rendevouz) x Apostle	A
Marksman	98ST08 x Aardvark	C
Mascot	Reaper x Rialto	C
Monty	Robigus x NFC10035	D
Oakley	Access x Robigus	B
Panorama	(Xi19 x Solstice) x Solstice	A
Paragon ¹	CSW 1724/19/6/68 x (Axona x Tonic)	ND
Qplus	Solstice x Robigus	D
Riband	Norman x (Maris Huntsman x TW161)	D
Robigus	Z836 x 1366	C
Scout	Z435 x Deben	C
Sherborne	Aardvark sib x Biscay	B
Shogun	Mallet x Whistler	C
Soissons	Jena x HN 35	C
Solstice	Vivant x Rialto	A
Viscount	Robigus x Canterbury	D
Walpole	Xi19 x Solstice	C
Welford	CWW 92/1 x FD92054	D
Xi19	(Cadenza x Rialto) x Cadenza	B
Zebedee	Claire x Nelson	A

¹ Limerick and Paragon were not included in the 2009 elite winter wheat and TAB field trial but were part of SSR marker analysis.

² A = low TAB, D = high TAB, ND = no data

Within the WGIN project Cadenza was identified as a consistently low take-all inoculum builder (McMillan et al., 2011) and two possible QTLs have since been identified in an Avalon x Cadenza doubled haploid mapping population (unpublished data, see Introduction to this chapter). The first pedigree investigated was therefore Cadenza (Figure 4.3). Cadenza is present in the pedigree of a number of National and Recommended List UK winter wheat varieties via Aardvark, Cordiale, Xi19 and

Scorpion 25 (Figure 4.3). In the WGIN field trials Cordiale and Xi19 were relatively low inoculum builders, indicating a possible inherited source of the low TAB trait. The current UK wheat variety Grafton, developed from Cadenza via Cordiale was also a low inoculum builder in the 2009 PhD field trial (Figure 4.3). There are five current elite varieties developed from Cadenza via Xi19. Three of these varieties, Bantam, Gallant and Panorama, were also low take-all inoculum builders in the 2009 trial. The other two varieties, Ketchum and Walpole had moderate take-all inoculum building scores. Battalion, Hyperion and Marksman developed from Cadenza via Aardvark, and Duxford developed via Scorpion 25, were medium to high inoculum builders in the 2009 field trial, perhaps indicating that the low TAB trait has not been inherited down these routes.

Other current wheat varieties in the 2009 PhD field trial exhibited the low TAB trait but had pedigrees unrelated to Cadenza. Another key variety investigated was Claire. The pedigree of Claire is depicted in Figure 4.4. Claire was investigated on the basis that this variety has been successfully used by the wheat breeders to develop four current elite wheat varieties in the 2009 trial, namely Alchemy, Cassius, Istabraq and Zebedee. Also some of the parents/grandparents of Claire have been quite widely used in wheat breeding programmes over the last three decades. Two notable side branches on the Claire tree have been included as these have led to the production of the current wheat varieties Hereford and Scout via Wasp (parent of Claire) and Einstein via Galahad (grandparent of Claire).

The four current varieties descended directly from Claire showed a range of TAB abilities in the 2009 field trial (09/R/WW/916), with Alchemy, Cassius and Zebedee as low to medium inoculum builders and Istabraq as a high take-all inoculum builder (Figure 4.4). Claire was also present in the WGIN field trials, so field performance data are available over 5 years (4 years in WGIN field trials and one year in the 2009 elite winter wheat variety PhD field trial). Claire was somewhat inconsistent over the years of WGIN field trials with a low TAB phenotype in 2005 (the presence of *Phialophora* sp. in the field in 2005 also inhibited take-all inoculum build-up under all varieties), 2008 and 2009, but a high take-all inoculum building performance in 2006 and 2007. This suggests a less stable phenotype than Cadenza, which has performed much more consistently.

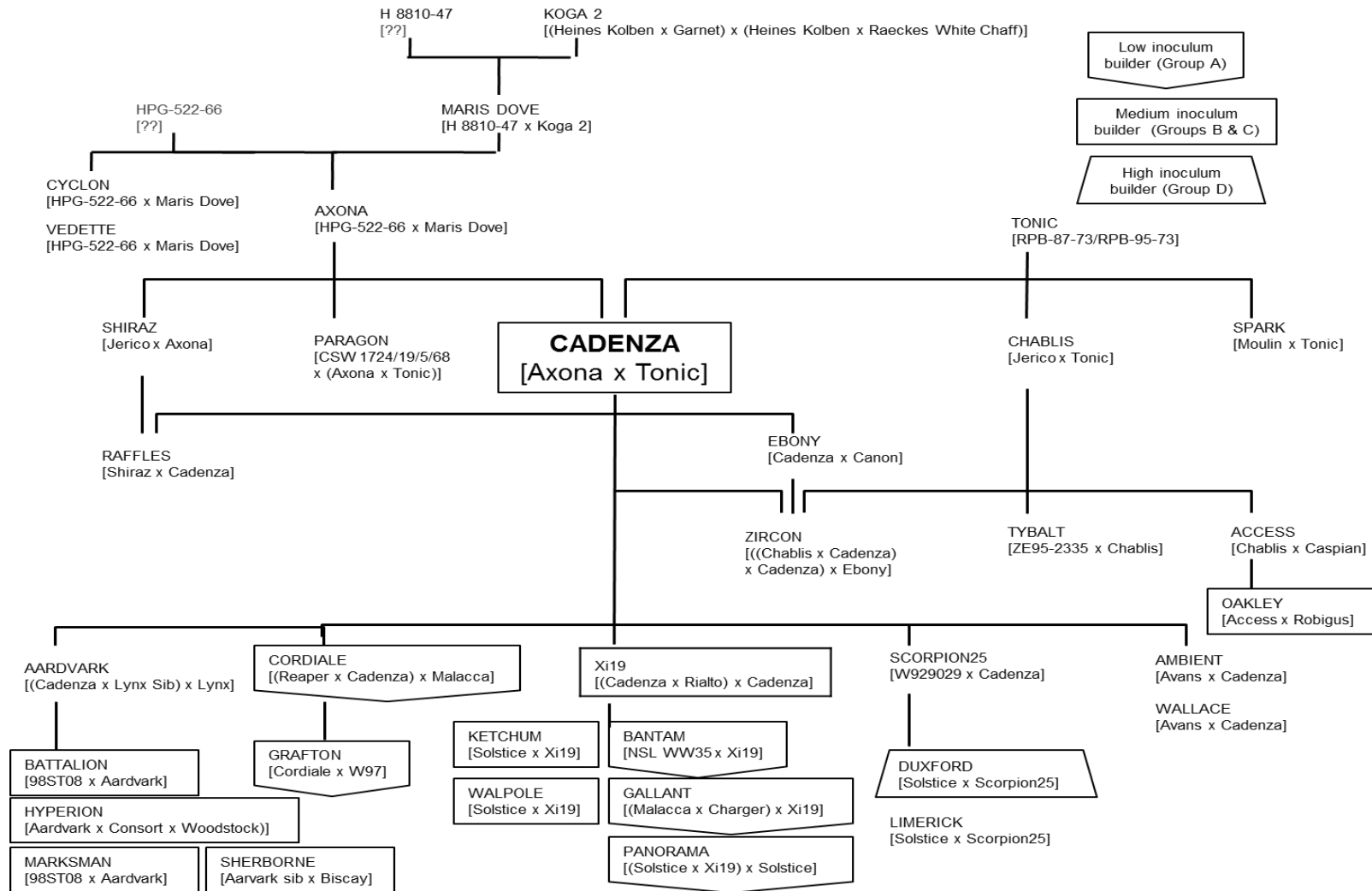


Figure 4.3. Cadenza family tree. Varieties within boxes were included in the 2009 elite winter wheat and TAB PhD trial. Varieties were divided into quartiles based on their TAB score and this is represented by box shape (see key at top right).

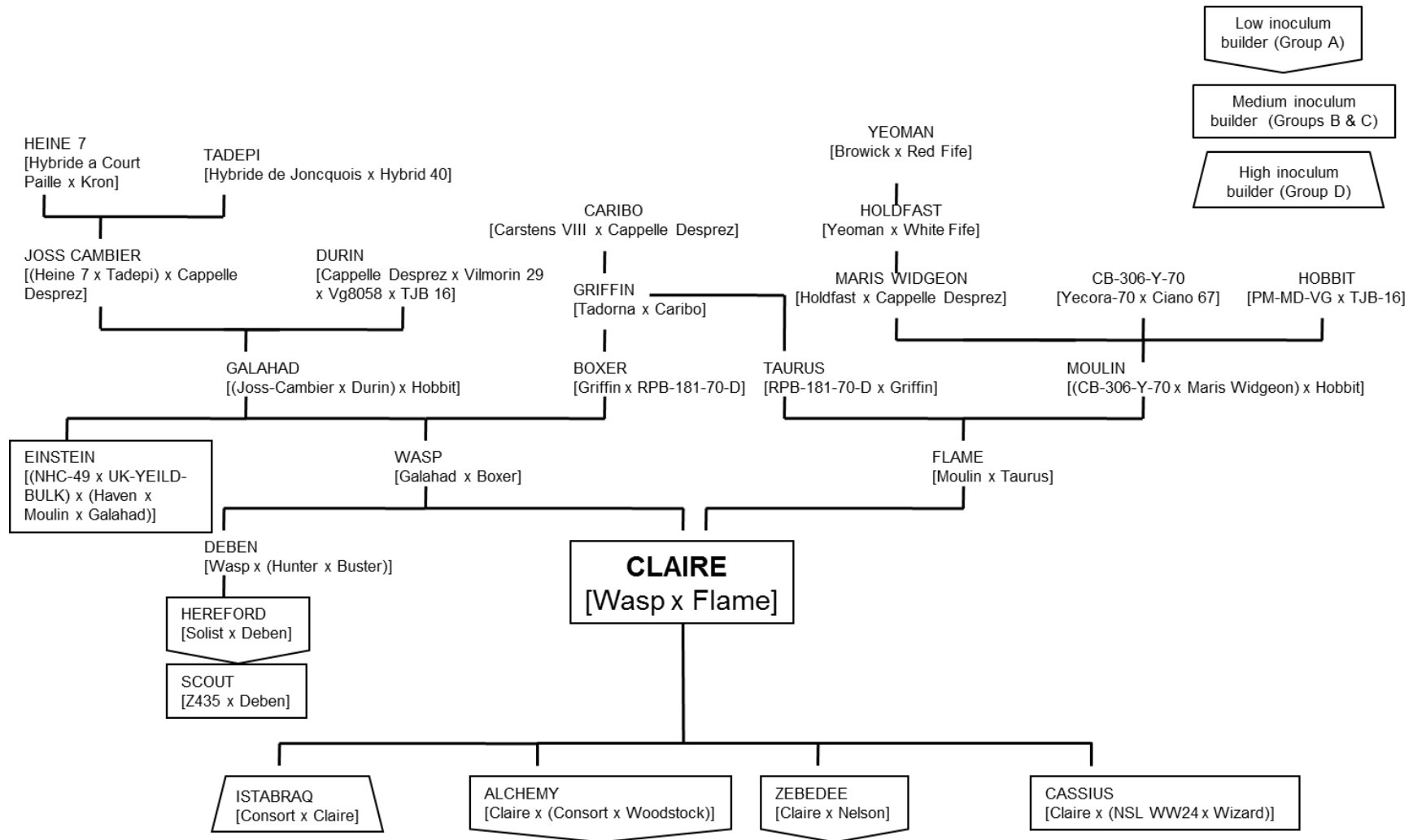


Figure 4.4. Claire family tree. Varieties within boxes were included in the 2009 elite winter wheat and TAB PhD trial. Varieties were divided into quartiles based on their inoculum build-up score and this is represented by box shape (see key at top right).

Sources of high TAB were also investigated. The pedigree of Avalon, the high TAB parent in the DH mapping population, was examined first (Figure 4.5). The eight elite wheat varieties with Avalon in their pedigree were all low or medium TAB varieties in the 2009 PhD field trial (09/R/WW/916), suggesting that the high TAB trait has not been inherited from Avalon. The other consistently high TAB variety in the WGIN field trials and PhD study was Hereward. However, Hereward has not been widely used in plant breeding and is not represented in the other elite wheat varieties in the PhD study. When examining the pedigrees of the 45 elite varieties in the 2009 PhD trial (Table 4.1) it was noticed that the variety Robigus was in the pedigrees of several varieties with high TAB scores in the field (Figure 4.6). Although Robigus itself was a generally low to medium building variety within the WGIN trials (McMillan et al., 2011). The results of the pedigree and TAB analysis of Cadenza, Claire, Avalon and Robigus are summarised in Table 4.2.

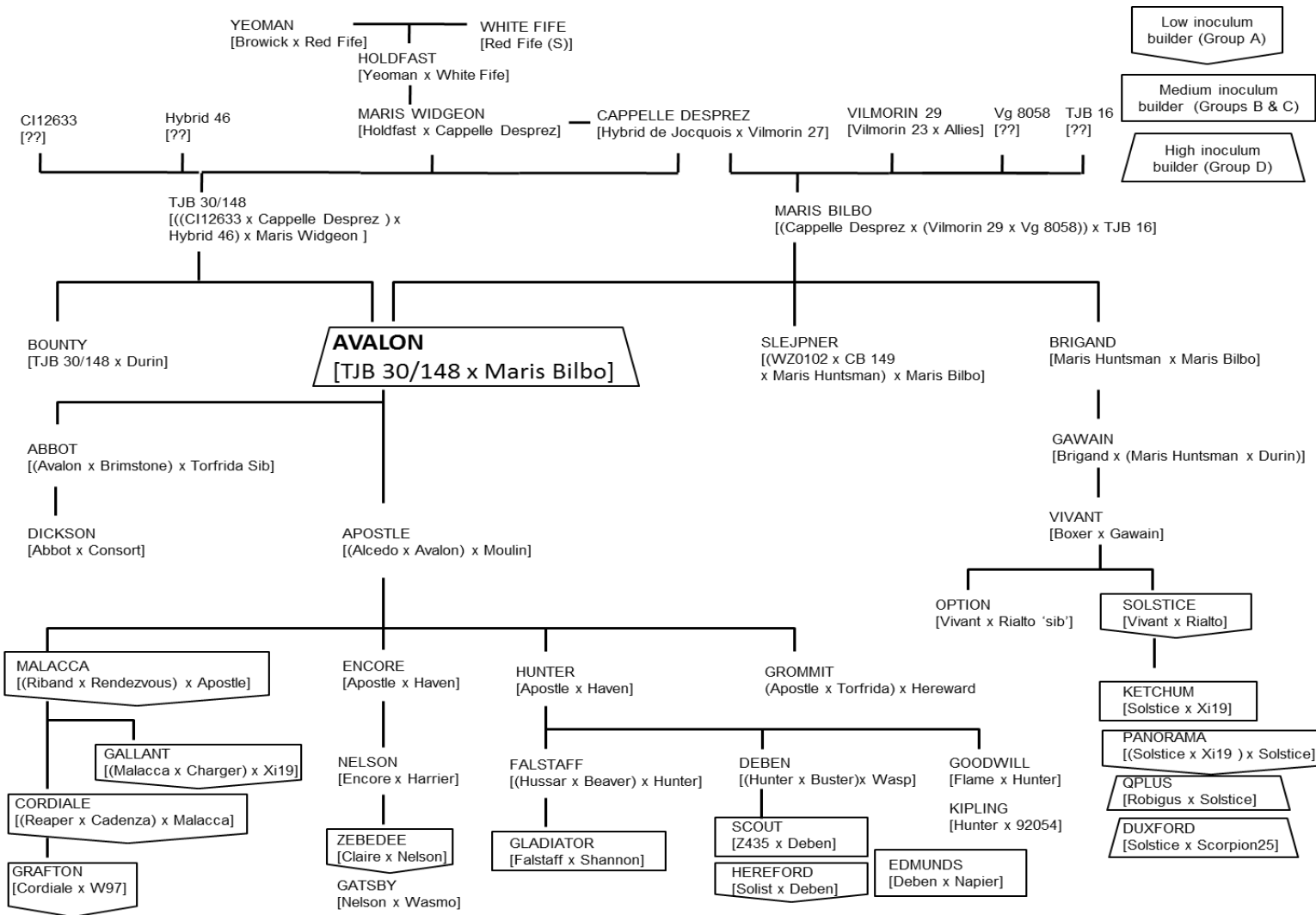


Figure 4.5. Avalon family tree. Varieties within boxes were included in the 2009 elite winter wheat and TAB PhD trial. Varieties were divided into quartiles based on their TAB score and this is represented by box shape (see key at top right).

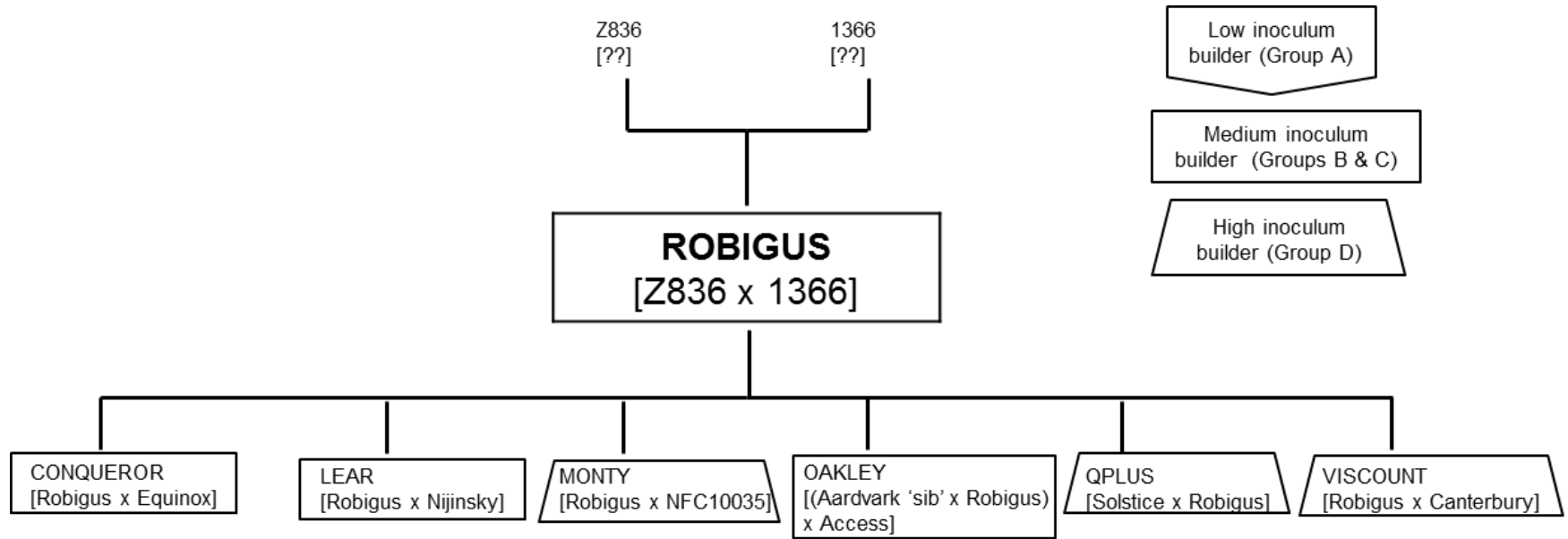


Figure 4.6. Robigus family tree. Varieties within boxes were included in the 2009 elite winter wheat and TAB PhD trial. Varieties were divided into quartiles based on their TAB score and this is represented by box shape (see key at top right).

Table 4.2. Inoculum building ability and pedigree information of the elite winter wheat varieties in the 2009 PhD field trial. Varieties have been divided into quartile groups (A-D) based on the percentage of bait plant roots infected in the soil core bioassay used to measure take-all inoculum build-up in the field (Trial 09/R/WW/916, see Chapter 3). Varieties in red and bold indicate those varieties with Cadenza in their pedigree as a parent or grandparent, varieties in blue and bold have Avalon in their pedigree, varieties in orange and bold have Claire in their pedigree, and varieties in purple and bold have Robigus in their pedigree. Varieties in half one colour and half another have two varieties in their pedigree.

TAB quartile group (mean % roots infected)			
A (50.6%)	B (58.2%)	C (66.3%)	D (72.1%)
Alchemy	Battalion	Conqueror	Avalon
Bantam	Cadenza	Einstein	Brompton
Cordiale	Cassius	Hyperion	Duxford
Gallant	Claire	Kipling	Hereward
Grafton	Edmunds	Marksman	Istabraq
Hereford	Gladiator	Mascot	JB Diego
Invicta	Humber	Robigus	Monty
Malacca	Ketchum	Scout	Qplus
Panorama	Lear	Shogun	Riband
Solstice	Oakley	Soissons	Viscount
Zebedee	Sherborne	Walpole	Welford
	Xi19		

4.3.2. Investigation of the genetic basis of the TAB trait: SSR marker analyses

Forty-seven wheat varieties were genotyped with nine SSR markers distributed along the chromosome regions of interest which contain predicted major QTLs conferring the low TAB trait identified in the Avalon x Cadenza DH mapping population. The diagnostic band size for Cadenza (low TAB) and Avalon (high TAB) alleles at each marker position are shown in Figures 4.7 and 4.8. The marker alleles for the 47 varieties at the four loci on chromosome A and the five loci on chromosome B are shown in Appendix 4.4. Banding patterns were scored as the same as either Avalon or Cadenza or different. The arrows in each gel picture indicate the position of the Avalon and Cadenza diagnostic bands. The range of allele sizes explored for the nine markers was between 105 to 300 base pairs. The overall results are summarised in Tables 4.3, 4.4 and 4.5.

Some banding patterns were difficult to discriminate; sometimes because these lanes were further away from the positive control lanes and/or due to gel 'smiling'. This made it difficult to determine whether the band sizes were identical. Therefore some samples were re-run on further Li-Cor gels with the positive control(s) Cadenza and/or Avalon as shown in Appendix 4.4. Sometimes there was also quite low amplification of microsatellite DNA sequences for some samples, represented by very weak fluorescent bands. In this case these samples were re-run with a lower dilution of PCR product to Li-Cor loading dye.

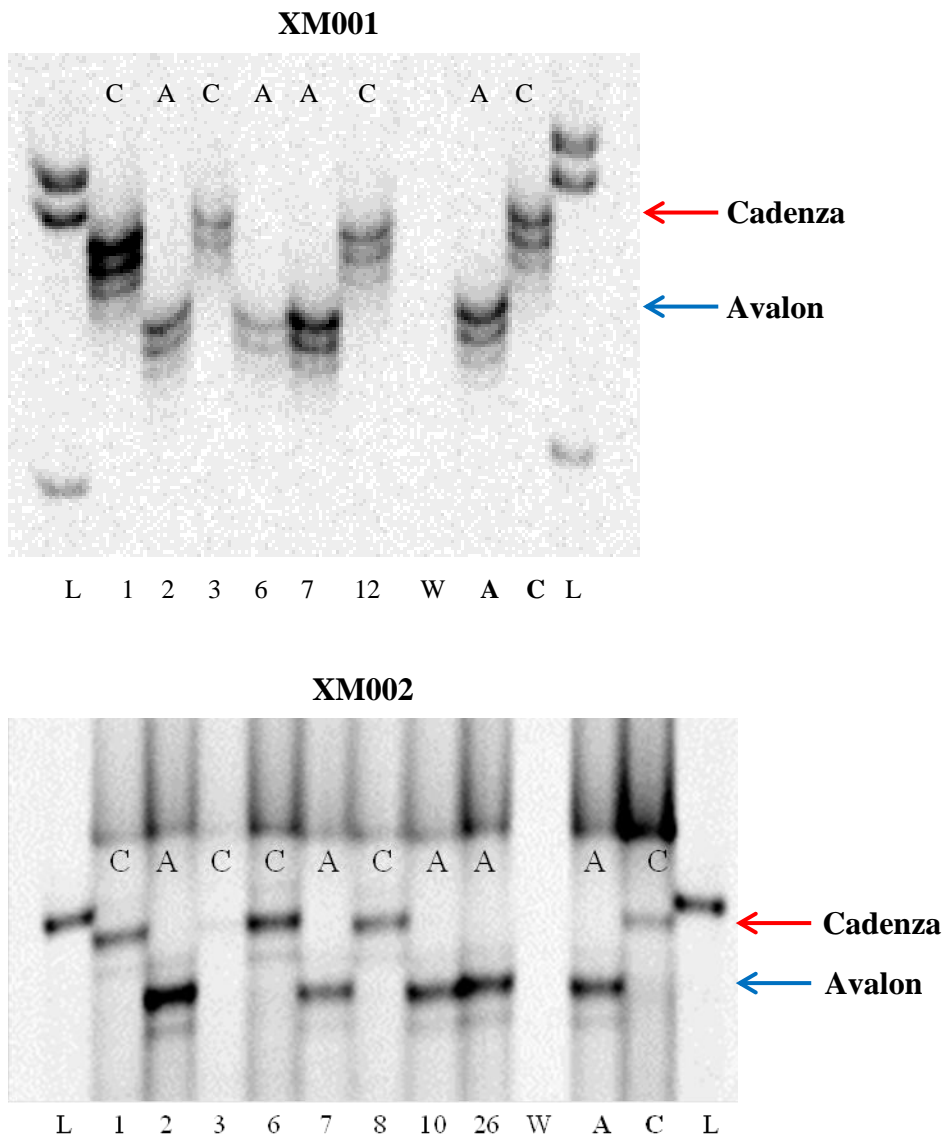
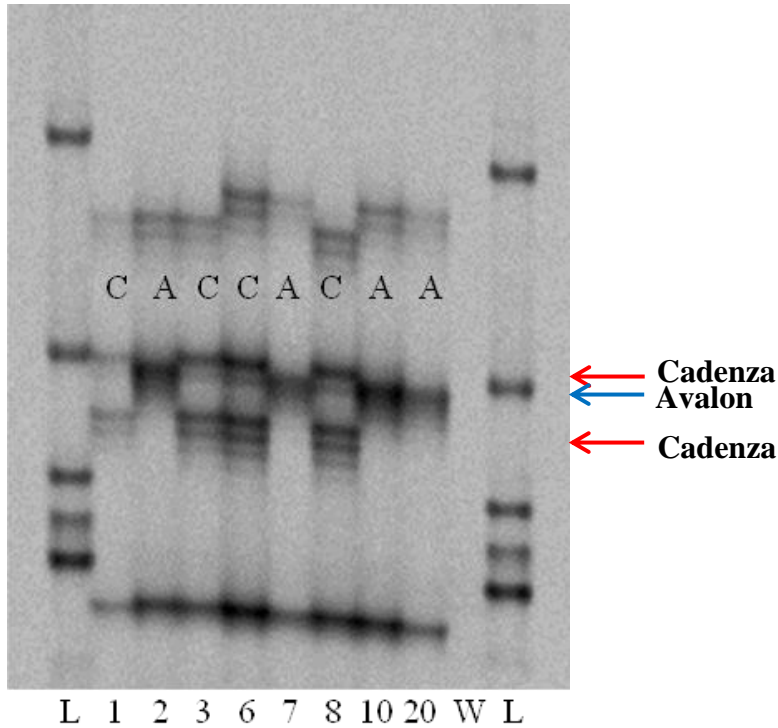


Figure 4.7. Marker alleles at XM001, XM002 (this page), XM003 and XM004 (next page) loci on chromosome A. AxC line numbers are shown along the bottom of each picture; A = Avalon parent; C = Cadenza parent. L = size ladder. W = water control. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

XM003



XM004

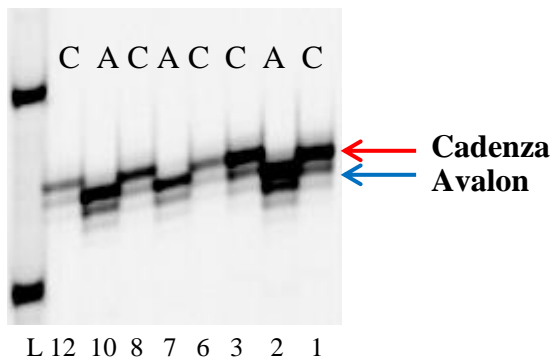
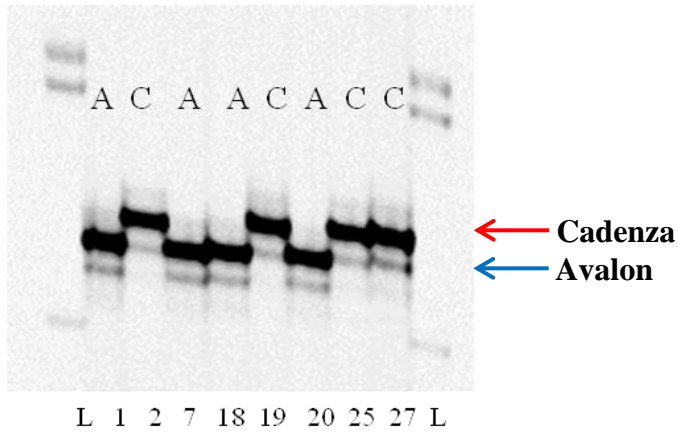
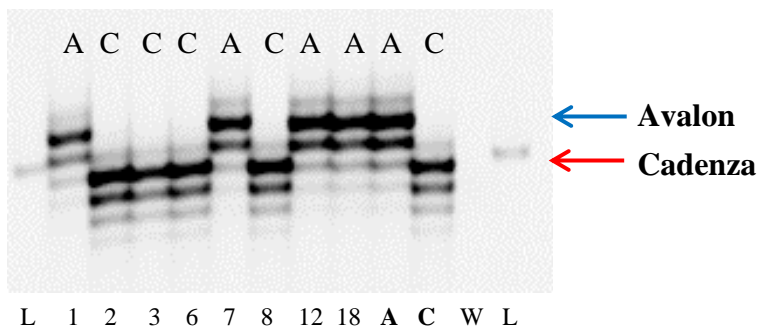


Figure 4.7. Marker alleles at XM001, XM002 (previous page), XM003 and XM004 (this page) loci on chromosome A. AxC line numbers are shown along the bottom of each picture; A = Avalon parent; C = Cadenza parent. L = size ladder. W = water control. Arrows indicate the position of diagnostic bands for Cadenza and Avalon. The Cadenza allele at loci XM003 is represented by two PCR bands.

XM005



XM006



XM007

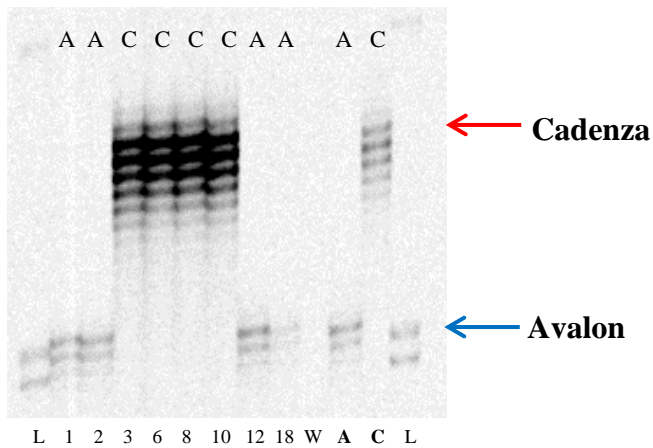


Figure 4.8. Marker alleles at XM005, XM006, XM007 (this page), XM008 and XM009 (next page) loci on chromosome B. AxC line numbers are shown along the bottom of each picture; A = Avalon parent; C = Cadenza parent. L = size ladder. W = water control. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

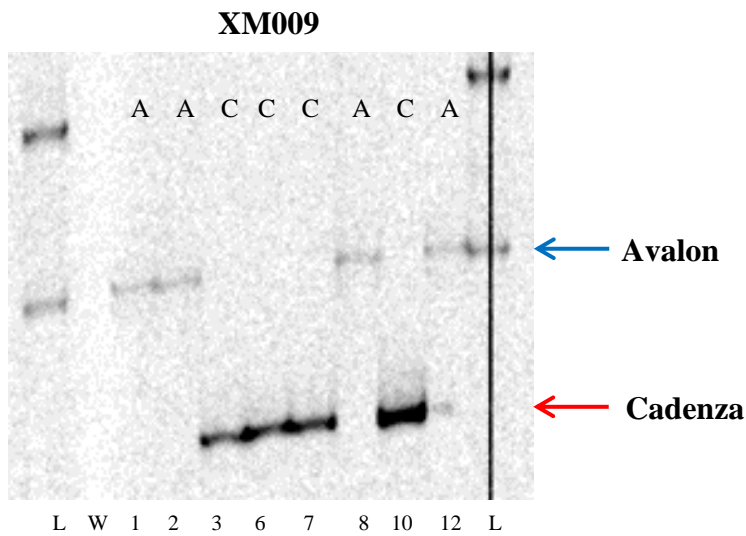
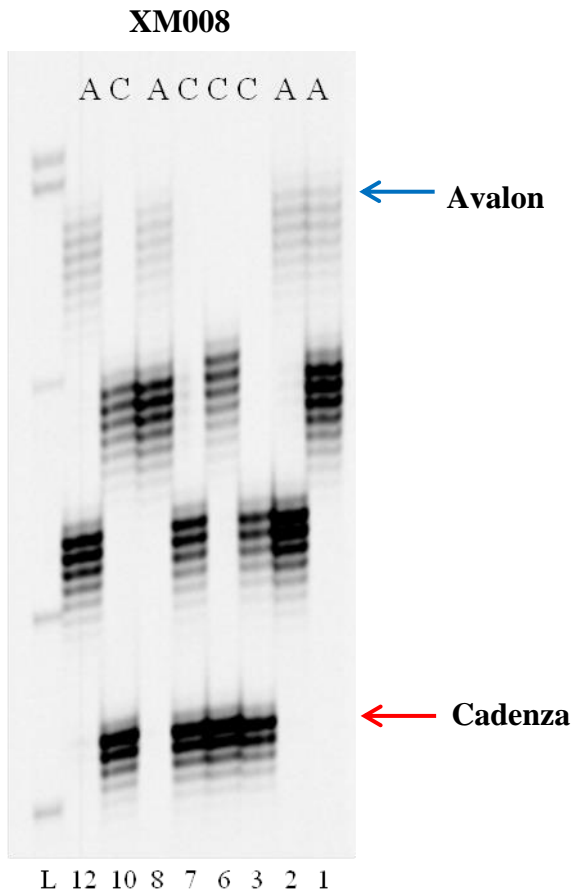


Figure 4.8. Marker alleles at XM005, XM006, XM007 (previous page), XM008 and XM009 (this page) loci on chromosome B. AxC line numbers are shown along the bottom of each picture; A = Avalon parent; C = Cadenza parent. L = size ladder. W = water control. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

Initially only the two markers that were most closely associated with the low TAB trait at the putative QTLs (M004 and M008), were selected for the study. Four varieties (Malacca, Paragon, Riband and Xi19) had the same alleles as Cadenza at both of these marker loci (Table 4.3). The pedigrees of Malacca, Xi19 and Paragon are all related to Cadenza, while Riband is not related. Twelve of the varieties had only one of the marker alleles in common with Cadenza; four varieties at XM004 on chromosome A and eight varieties at XM008 on chromosome B. Only one variety, Edmunds, had both marker alleles the same as Avalon (Table 4.4). The pedigree of Edmunds is related to Avalon via Apostle, Hunter and Deben (Figure 4.5). Eleven varieties had only one marker allele the same as Avalon; eight varieties at XM004 and three varieties at XM008 (Table 4.4).

In the 2009 elite winter wheat and TAB field trial (09/R/WW/916), the varieties which were found to contain Cadenza alleles at either XM004 or XM008 or both these marker loci were present in both low and high inoculum building quartiles (Groups A, B and D; Table 4.3). Varieties with the Avalon alleles at either XM004 or XM008 or at both these marker loci were found in all four quartile groups (Table 4.4). This indicates that neither M004 nor M008 or a combination of these two markers is able to accurately identify the low TAB trait containing varieties. Within the 2009 field trial there were 14 descendants of Cadenza which are shown in bold text in Tables 4.3 and nine descendants of Avalon shown in bold text in Table 4.4. There are also five other varieties (Duxford, Ketchum, Panorama, QPlus and Solstice) that are related to Avalon via the parent of Avalon, Maris Bilbo (Figure 4.5). Eleven of these varieties have the Cadenza or Avalon allele at one or both of the marker loci. Other varieties containing the Cadenza or Avalon alleles at one or both of the marker loci are not directly related to Cadenza or Avalon and therefore the origin of their alleles is not known.

Table 4.3. Inoculum building ability and marker allele comparison between the low TAB variety Cadenza and other elite winter wheat varieties. Varieties have been divided into quartile groups (A-D) based on the percentage of bait plant roots infected in the soil core bioassay used to measure inoculum build-up in the field (Trial 09/R/WW/916, see Chapter 3). Bold = varieties with Cadenza in their pedigree.

TAB quartile group (mean % roots infected)			
A (50.6%)	B (58.2%)	C (66.3%)	D (72.1%)
Alchemy	Battalion	Conqueror	Avalon
Bantam	Cadenza	Einstein	Brompton
Cordiale	Cassius	Hyperion	Duxford
Gallant	Claire	Kipling	Hereward
Grafton	Edmunds	Marksman	Istabraq
Hereford	Gladiator	Mascot	JB Diego
Invicta	Humber	Robigus	Monty
Malacca	Ketchum	Scout	Qplus
Panorama	Lear	Shogun	Riband
Solstice	Oakley	Soissons	Viscount
Zebedee	Sherborne	Walpole	Welford
	Xi19		

	Both Cadenza alleles
	Cadenza allele at XM004 (Chromosome A)
	Cadenza allele at XM008 (Chromosome B)

Table 4.4. Inoculum building ability and marker allele comparison between the high TAB variety Avalon and other elite winter wheat varieties. Varieties have been divided into quartile groups (A-D) based on the percentage of bait plant roots infected in the soil core bioassay used to measure inoculum build-up in the field (Trial 09/R/WW/916, see Chapter 3). Bold = varieties with Avalon in their pedigree.

TAB quartile group (mean % roots infected)			
A (50.6%)	B (58.2%)	C (66.3%)	D (72.1%)
Alchemy	Battalion	Conqueror	Avalon
Bantam	Cadenza	Einstein	Brompton
Cordiale	Cassius	Hyperion	Duxford
Gallant	Claire	Kipling	Hereward
Grafton	Edmunds	Marksman	Istabraq
Hereford	Gladiator	Mascot	JB Diego
Invicta	Humber	Robigus	Monty
Malacca	Ketchum	Scout	Qplus
Panorama	Lear	Shogun	Riband
Solstice	Oakley	Soissons	Viscount
Zebedee	Sherborne	Walpole	Welford
	Xi19		

	Both Avalon alleles
	Avalon allele at XM004 (Chromosome A)
	Avalon allele at XM008 (Chromosome B)

After the initial marker study with M004 and M008 the number of markers tested was increased to look at the genetic similarity of varieties across the two QTL regions (Table 4.5). All of the varieties had a least two marker alleles in common with Avalon and Cadenza at the nine marker loci tested. Only the variety Xi19, a direct descendant of Cadenza, had the same marker alleles as Cadenza at all nine markers tested. Paragon also had the same marker alleles as Cadenza, except for at one marker loci, XM001, on chromosome A. Paragon is the product of a cross between the same parents as Cadenza, Axona and Tonic, and a third coded line, CSW 1724/19/6/68. The variety Xi19 was a consistently low TAB variety in the WGIN and PhD field trials but Paragon was a medium to high building variety in the two years of WGIN trials it was sampled in (2007 and 2008) (McMillan et al., 2011). The variety Duxford has the same alleles as Cadenza at chromosome A, but not chromosome B (Table 4.5). Duxford was one of the highest building varieties in the 2009 field trial (Table 4.3). No varieties had the same marker alleles as Avalon at all of the nine marker loci.

Table 4.5. Microsatellite allele comparison between the mapping population parent varieties, Avalon and Cadenza, and other elite winter wheat varieties used in the PhD study. Red and bold = loci most closely associated with low TAB trait in QTL analysis. A = allele same as Avalon; C = allele same as Cadenza; x = allele different to Avalon and Cadenza; - = null allele.

Code	Variety	Chromosome A				Chromosome B					TAB ¹
		Position (cM)				Position (cM)					
		10	24	26	34	51	59	82	92	112	
	XM001	XM002	XM003	XM004	XM005	XM006	XM007	XM008	XM009		
1	Alchemy	A	x	x	C	A	x	x	x	x	A
2	Avalon	A	A	A	A	A	A	A	A	A	D
3	Bantam	C	C	C	x	x	x	x	C	A	A
4	Battalion	A	A	x	x	x	A	x	x	C	B
5	Brompton	A	A	x	x	x	x	A	x	C	D
6	Cadanza	C	C	C	C	C	C	C	C	C	B
7	Cassius	A	x	x	x	x	A	x	x	C	B
8	Claire	C	x	x	x	C	A	x	x	x	B
9	Conqueror	x	C	x	x	x	A	C	C	A	C
10	Cordiale	A	A	x	A	C	A	C	x	x	A
11	Duxford	C	C	C	C	x	A	C	x	C	D
12	Edmunds	A	x	x	A	x	A	x	A	A	B
13	Einstein	A	x	x	A	x	A	C	C	C	C
14	Gallant	A	x	x	A	C	A	C	C	C	A
15	Gladiator	A	x	x	A	x	A	C	x	A	B
16	Grafton	A	x	x	x	C	A	A	x	x	A
17	Hereford	x	x	x	x	A	x	-	C	C	A
18	Hereward	x	A	x	A	C	x	C	x	x	D
19	Humber	A	x	x	x	x	x	C	A	-	B

Table 4.5. Continued

Code	Variety	XM001	XM002	XM003	XM004	XM005	XM006	XM007	XM008	XM009	TAB ¹
20	Hyperion	A	A	x	x	x	A	A	x	x	C
21	Invicta	x	C	x	x	A	A	x	x	x	A
22	Istabraq	C	x	x	x	A	A	x	C	C	D
23	JB Diego	A	x	x	C	x	x	A	A	x	D
24	Ketchum	A	x	x	x	x	A	C	x	C	B
25	Kipling	A	A	x	A	x	A	x	x	x	C
26	Lear	C	C	x	x	x	A	A	x	x	B
27	Malacca	A	A	x	C	C	x	x	C	C	A
28	Marksman	A	x	x	A	x	A	C	x	x	C
29	Mascot	A	x	x	A	x	A	C	x	C	C
30	Monty	x	C	x	x	x	A	x	x	x	D
31	Oakley	x	C	x	x	x	A	x	x	x	B
32	Panorama	A	x	x	x	x	A	C	C	C	A
33	Q Plus	A	C	x	x	x	A	C	x	x	D
34	Riband	A	x	x	C	A	A	x	C	C	D
35	Robigus	x	C	x	x	x	A	x	x	x	C
36	Scout	C	C	x	x	x	A	x	A	A	C
37	Sherborne	x	C	x	x	x	A	x	x	x	B
38	Shogun	A	x	x	x	x	A	C	x	x	C
39	Soissons	A	x	x	x	A	x	x	x	x	C
40	Solstice	A	x	x	x	x	A	C	x	C	A
41	Viscount	A	C	x	x	x	A	x	x	x	D
42	Walpole	A	C	C	x	x	A	C	C	C	C
43	Welford	A	x	x	C	x	x	A	x	C	D
44	Xi19	C	C	C	C	C	C	C	C	C	B

Table 4.5. Continued

Code	Variety	XM001	XM002	XM003	XM004	XM005	XM006	XM007	XM008	XM009	TAB ¹
45	Zebedee	A	x	x	x	x	A	x	x	x	A
46	Limerick	A	x	x	x	x	A	x	x	C	-
47	Paragon	x	C	C	C	C	C	C	C	C	-

¹ Take-all inoculum build-up in the 2009 PhD field trial (09/R/WW/916). Varieties have been divided into quartile groups based on the percentage of bait plant roots infected in the soil core bioassay used to measure take-all inoculum in the field. A = low TAB, D = high TAB, - = not included in 2009 trial.

4.4. Discussion

In this study pedigree and SSR analyses were carried out to investigate the genetic basis of the TAB trait within UK elite winter wheat varieties. Caution must be taken when analysing the results as the TAB phenotype of varieties was generally only based on one year of data from the 2009 elite winter wheat and TAB field trial (09/R/WW/916; Chapter 3). Work is also ongoing within WGIN programme and BBSRC-TSB LowTAB project to further confirm the location of the two QTLs.

In the initial SSR marker analysis the two markers most associated with the TAB trait were used to screen the elite wheat varieties. The presence of the Cadenza or Avalon alleles at these markers was not related to TAB phenotype in the field, suggesting that these markers are either not tightly linked to the QTL, or that other genetic regions are important in the different genetic backgrounds of the elite wheat varieties tested. Either way these markers were not diagnostic for the low TAB trait. The SSR banding patterns of the elite winter wheat varieties were then compared with those of Cadenza and Avalon with a selection of other markers across the whole putative QTL regions. This demonstrated that no variety except for Xi19 had the same alleles as Cadenza at all nine marker loci tested, suggesting that only Xi19 has both of the complete putative QTL regions conferring low TAB. Xi19 is closely related to Cadenza, arising from a three way cross, where Cadenza was used as both the initial female parent, and then in the backcross. Xi19 has displayed a consistently low TAB phenotype in the WGIN field trials and the PhD trial in 2009. The other low to medium TAB varieties with Cadenza in their pedigree did not contain the same marker alleles as Cadenza across the QTL regions, suggesting either that the two QTLs are not conferring the low TAB trait in these varieties or that the other parents of these varieties are contributing regions on chromosomes A and B for low TAB.

Two varieties contained the same alleles as Cadenza across either one of the QTL regions; Duxford across chromosome A and Paragon across chromosome B. The pedigrees of both Duxford and Paragon are related to Cadenza. These two varieties have both been medium to high take-all inoculum builders in the 2009 PhD field trial (Duxford) and WGIN trials (Paragon) perhaps suggesting that the presence of only one QTL region is not sufficient to limit build-up. Alternatively, due to the relatively large genetic distances between some SSR markers it is possible that recombination may have occurred between markers in these varieties, so that the QTL region is not intact. As the putative QTL regions have also yet to be confirmed in further field trials with the full

set of 204 A x C lines it is possible that there could be a large environmental effect and the QTLs are not stably expressed across sites and seasons. None of the current varieties tested had all of the same alleles as the older variety Avalon. The pedigree and SSR marker work suggest that there are likely to be multiple unrelated genetic sources of both low and high TAB within the current UK elite winter wheat breeding pool, as low and high TAB varieties did not have SSR alleles similar to those characterised in Cadenza or Avalon, respectively.

In the pedigree analysis Claire was identified as another source of the low TAB trait while Robigus was implicated as a source of the high TAB trait. Robigus was a common parent occurring in the parentage of three of the very highest inoculum builders in the 2009 field trial (Monty, Qplus and Viscount). Robigus itself has been more of a low to moderate take-all inoculum builder in the WGIN field trials so the high TAB trait in its direct descendants may be due to the influence of other parents in the crosses. To further extend these pedigree analyses, seed for key varieties in each of the pedigrees of highest interest has been obtained, particularly in the Cadenza and Avalon pedigrees. In total 88 additional varieties have been procured. In the future these additional varieties will be screened in the SSR analyses to trace the origins of the TAB trait. Each variety has also been grown, in a protected greenhouse over the winter, to bulk up seed stocks for future screening for the TAB trait in the field. This work has not been possible within the timeframe of my PhD but will be taken forward within the take-all research group at Rothamsted.

CHAPTER 5: CHARACTERISATION OF A NEW *Ggt* ISOLATE COLLECTION

5.1. Introduction

Population genetic studies using molecular DNA tools have commonly identified two major sub-populations of *Ggt* in the field (Daval et al., 2010). The approaches used have included Restriction Fragment Length Polymorphism (RFLP) (Bateman et al., 1992, O'Dell et al., 1992, Tan et al., 1994, Bateman et al., 1997), Random Amplified Polymorphic DNA (RAPD) (Augustin et al., 1999, Bryan et al., 1999, Irzykowska & Bocianowski, 2008) and Amplified Fragment Length Polymorphism (AFLP) (Lebreton et al., 2004) fingerprinting. At Rothamsted, Freeman et al. (2005) also developed a specific PCR assay to detect *Ggt* and differentiate between two sub-populations. Sub-populations have been characterised differently as A/B (Freeman et al., 2005), G₁/G₂ (Lebreton et al., 2004), N/R (O'Dell et al., 1992, Bryan et al., 1999), T1/T2 (Bateman et al., 1992) and A1/A2 (Augustin et al., 1999). A smaller number of studies have found more than two main groupings of *Ggt* isolates, indicating a higher level of inter-varietal variation. In Poland Irzykowska and Bocianowski (2008) identified many small sub-groups of *Ggt* based on analysis of RAPD data. In Australia Tan et al. (1994) detected three subgroups of *Ggt* (T1/T2/T3) based on ribosomal DNA sequences.

The two main genetic groups of *Ggt* have been associated with biological features. Lebreton et al. (2007) found that the relative proportions of their G₁/G₂ sub-populations were correlated with different stages of the take-all epidemic in cereal sequences. G₁ isolates were most common in the first wheat crop, while G₂ isolates peaked in number in the 3rd or 4th crop. Approximately equal amounts of G₁ and G₂ isolates were then found in the 5th wheat crop onwards. Disease severity has also been linked to isolate genotype (Bateman et al., 1997, Lebreton et al., 2007, Willocquet et al., 2008). Bateman et al. (1997) also found that the proportion of their T1 and T2 isolates differed depending on whether the cereal crop was wheat or barley. While the N/R isolate groups have been associated with their ability to infect rye, R type isolates being able to infect and N isolates not able to infect (Bryan et al., 1999).

Until recently there was little information on the degree of similarity between the two main *Ggt* sub-populations identified using the different techniques; generally the sub-populations were identified from different isolate collections and geographical regions (Daval et al., 2010). A comparison of isolate genotypes was carried out by Daval et al. (2010) using two PCR tools to detect G₁/G₂ and A/B on a worldwide collection of 98

Ggt isolates. The isolates used had previously been partly characterised as A1/A2, A/B, G₁/G₂, R/N or T1/T2 by different research groups around the world. This study demonstrated that there was a strong relationship between the methods previously used, indicating that there are two major genetic *Ggt* sub-populations worldwide. Isolates identified as B type, G₁, R and T2 or A, G₂, N and T1 were very strongly correlated. There were also a small number of isolates that did not show a correlation between the different molecular tools.

The aim of the work in the PhD study was to build up and characterise a new *Ggt* culture collection for use throughout the PhD in pot screening work of wheat germplasm for susceptibility to take-all (Chapters 6 and 7). The specific PCR assay developed by Freeman et al. (2005) was used to differentiate isolates collected into A or B type. *In vitro* fungicide sensitivity tests were also carried out to characterise the isolates as sensitive or resistant to the fungicide silthiofam. As described in Chapter 1 silthiofam (commercial name: Latitude®) is one of two commercially available seed treatment fungicides against take-all, the other being fluquinconazole (commercial name: Jockey®). A natural range of sensitivities of *Ggt* isolates to silthiofam is found in the field, with some isolates being completely resistant (Carter et al., 2003). In contrast Bateman et al. (2003) found no *Ggt* isolates that were resistant to fluquinconazole in four years of field trials treated with fluquinconazole. Previously Freeman et al. (2005) demonstrated a possible relationship between sensitivity to the fungicide silthiofam *in vitro* and polymorphisms in ITS2 of the nuclear rDNA as identified in the PCR assay to detect A/B type isolates. A comparison was therefore made in this study between the genetic sub-population type and sensitivity to silthiofam of the isolates collected. Sensitivity to silthiofam was also tested on silthiofam treated wheat seed, as a comparison to sensitivity *in vitro* on silthiofam amended PDA plates.

After characterisation in the PCR assay and fungicide sensitivity tests a subset of the isolates were then chosen for pathogenicity testing to confirm their ability to cause disease. A selection of isolates, representative of the field population, could then be chosen for pot screening work (see Chapter 2, page 48, for pot test method).

5.2. Materials and Methods

5.2.1. *Ggt* isolations

In Autumn 2008 *Ggt* field isolates were obtained from the Rothamsted farm (Field: Bones Close; clay-loam soil with flints). Root pieces (100 pieces *c.* 1 cm long) were cut from the root systems of bioassay plants (cv. Hereward) grown in soil cores taken after harvest from a first wheat crop in Bones Close. The root pieces were surface sterilised for 5 min in sodium hypochlorite (1:5 dilution with sterile distilled water), tripled rinsed in sterile distilled water and blotted dry on filter paper in a sterile air flow hood. Root pieces were then placed on potato dextrose agar (PDA) amended with penicillin (20 U ml⁻¹) and streptomycin sulphate (20 µg ml⁻¹) (Penicillin-Streptomycin GIBCO™, Invitrogen), and incubated at 15°C for 4 days. Cultures resembling *Ggt* were then transferred using a sterilised needle to fresh PDA plates containing the same antibiotics. After incubation at 15°C for 1 week uncontaminated *Ggt* cultures were further subcultured onto fresh PDA without addition of antibiotics. Cultures were incubated at 15°C until mycelium covered the plate (*c.* 2 weeks) and were then stored at 4°C for future use.

5.2.2. DNA extraction and PCR using *Ggt* specific primers

A 1 cm² agar plug of each isolate was transferred into 15 ml LB broth (Lennox Broth Base, GIBCO; peptone, 10 g L⁻¹; yeast extract, 5 g L⁻¹; NaCl, 5 g L⁻¹), in a universal bottle. Cultures were incubated at room temperature on an orbital shaker at 250 rpm for 8 days. Cultures were then transferred in a sterile air flow hood onto Whatman 3 MM chromatography paper, separated from the agar plug and blotted dry. The mycelial cultures were then transferred into 2 ml eppendorf tubes and stored at -20°C before freeze drying and DNA extraction.

Mycelium was freeze-dried overnight in 2 ml eppendorf tubes and ground using a sterile metal rod before DNA extraction. DNA was extracted using a method modified from Fraaije et al. (1999), as described previously (Ward et al., 2005a). Nucleic acids were extracted with the addition of 0.6 ml DNA extraction buffer (Appendix 5.1) and incubated at 70°C for 30 minutes in a hot block. Samples were vortexed before addition of 0.3 ml cold (-20°C) 7.5 M ammonium acetate. Samples were then incubated on ice for 30 minutes before centrifugation at 14,000 rpm for 10 minutes. The supernatant was transferred to fresh eppendorf tubes containing an equal volume of cold (-20°C) isopropanol. Samples were further incubated at -20°C for 2 hours before centrifugation

at 14,000 rpm for 10 minutes. The isopropanol supernatant was discarded and pelleted DNA was washed twice in cold (-20°C) 70% ethanol. Samples were mixed by inversion 15 times and centrifuged at 14,000 rpm for 3 minutes. The ethanol was discarded and nucleic acid pellets were air dried before resuspension in 100 µl TE. DNA extracts were stored at -20°C until future use.

PCR amplification using three primers Ggtfwd, GgtArev and GgtBrev2 (all used in a single PCR) was carried out to identify two genetic subpopulations (A and B) of *Ggt* as described by Freeman et al. (2005). PCR amplifications were carried out in 12.5 µl reaction mixtures containing 10x buffer (50 mM KCl, 10 mM Tris-HCl; Promega), 0.2 mM dNTP mix, 1 mM MgCl₂, 5 pmol of each of the three primers and 0.125 units of GoTaq® Flexi DNA polymerase (Promega) and DNA (1 µl 1 : 100 dilution of genomic DNA stock solution). Positive controls included one A type *Ggt* isolate (92.15.4A) and one B type *Ggt* isolate (99S9.4B) previously identified by Freeman et al. (2005). Primer sequences and PCR conditions used are described in Table 5.1. PCR products were resolved on 2% agarose gels with 0.25 µg ml⁻¹ ethidium bromide. Gels were run for 1½ hours at 80 V and visualised under UV light.

Table 5.1. PCR primer sequences and PCR conditions for identification of A/B type *Ggt* isolates.

Primer	Sequence (5'-3')	Product size (bp)	Thermocycling programme
Ggtfwd	AAG AAC ATC GGC GGT CTC GCC		Touchdown PCR with annealing temp range 72-67°C, decreasing by 1°C every 2 cycles; 20 cycles at min temp of 67°C
GgtArev	TAG CGG CTG GAG CCC GCC G	Ggtfwd & GgtArev: 93 bp	
GgtBrev2	CTA CCT GAT CCG AGG TCA ACC TAA GG	Ggtfwd & GgtBrev2: 132 bp	10 min at 94°C, followed by 30 cycles of: 30 sec at 94°C, annealing as described above, 1 min at 72°C, and final extension for 10 min at 72°C

5.2.3. Fungicide sensitivity testing

All isolates were tested for their sensitivity to silthiofam (commercial fungicide name: Latitude®). Sensitivity to silthiofam was tested on silthiofam amended PDA plates as described by Freeman et al. (2005). Silthiofam, formulated at the recommended dose for commercial seed treatment applications (125 g L^{-1}), was diluted in sterile distilled water and added to sterile, molten PDA to give a final concentration of 1 mg L^{-1} . Petri dishes of silthiofam amended PDA and control plates of unamended PDA were poured (c. 30 ml PDA in each). Three 6-mm-diameter agar discs (cut using a sterile cork borer, size 3) from the edges of colonies of *Ggt* were placed on each plate. Three replicate agar discs of each isolate were placed on three different plates for silthiofam amended PDA and unamended PDA. Plates were incubated at 21°C for four days and increases in colony diameter measured. Isolates were recorded as resistant to silthiofam if growth on the silthiofam amended PDA plates was $> 90 \%$ of that on unamended PDA plates.

5.2.4. Pathogenicity assay on wheat

A subset of *Ggt* isolates (10 silthiofam resistant and 10 silthiofam sensitive isolates) were tested in a wheat pathogenicity test to confirm the ability of the isolates to cause disease. Plastic pots (6 cm diameter by 10 cm deep) were filled with 120 cc moist sand. *Ggt* colonised agar ($\frac{1}{3}$ of a 9-cm Petri dish for each pot) was macerated and mixed with 50 cc sand and this mixture added as a layer of inoculum on top of moist sand in the plastic pots. Control pots contained non-colonised PDA. The pots were then topped up with a further 80 cc moist sand over the layer of inoculum. Five wheat seeds (cv. Hereward) were placed on the sand and covered with coarse horticultural grit. Silthiofam sensitivity was also tested using silthiofam treated wheat seed to confirm the results of the *in vitro* fungicide sensitivity test. Four pots per isolate (2 replicates with silthiofam treated seed and 2 replicates with untreated seed) were set up. All pots were placed in a controlled environment room in a randomised design (16 hour day, 70% RH, day/night temperatures $15/10^\circ\text{C}$, twice weekly watering). After 5 weeks the plants were removed and their roots washed out with water before disease assessment. Plant root systems were assessed for take-all lesions in a white dish under water. The percentage of roots infected with take-all was calculated as a measure of pathogenicity.

5.3. Results

5.3.1. Isolate characterisation by Ggt-specific PCR

A total of 40 *Ggt* isolates were obtained from 100 root isolations (Appendix 5.2). The collection of 40 newly isolated *Ggt* cultures were all positively classified into two genetic sub-populations (A- & B-type isolates) using a *Ggt* specific PCR (Figures 5.1 & 5.2). The majority of isolates (36/40) were classified as A-type isolates.

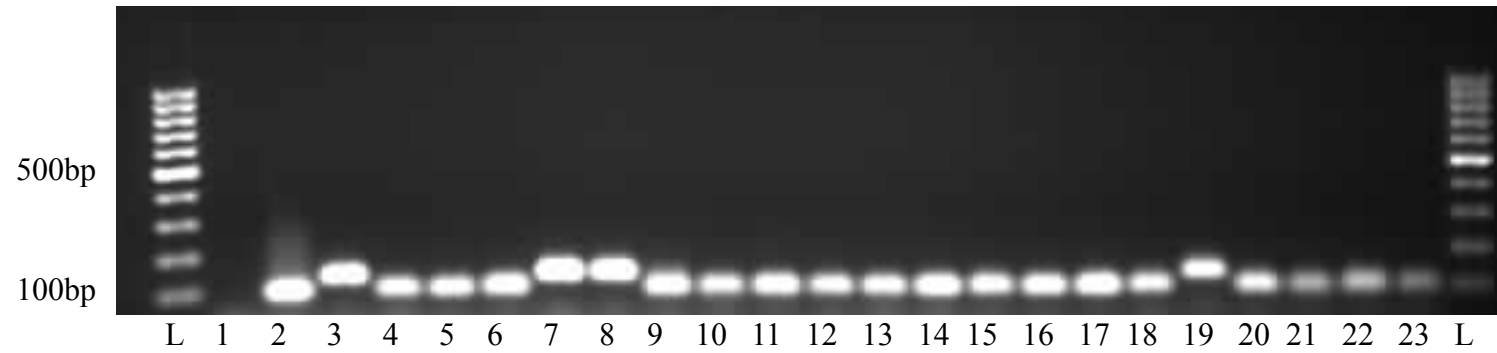


Figure 5.1. *Ggt* specific PCR analysis of 20 new *Ggt* isolates (BC01-BC20) obtained from root isolations of soil bioassay plants using primers Ggtfwd, GgtArev and GgtBrev2 to identify A and B *Ggt* subpopulations. Lane L, 100 bp DNA ladder (Promega); lane 1, no DNA; lane 2, positive control, *Ggt* A type isolate (92.15.4A), band size 93 bp; lane 3, positive control, *Ggt* B type isolate (99S9.4B), band size 132 bp; lanes 4-6, 9-18 and 20-23, *Ggt* A type isolates BC01-BC03, BC06-BC15 and BC17-BC20, respectively; lanes 7-8 and 19, *Ggt* B type isolates BC04-BC05 and BC16, respectively.

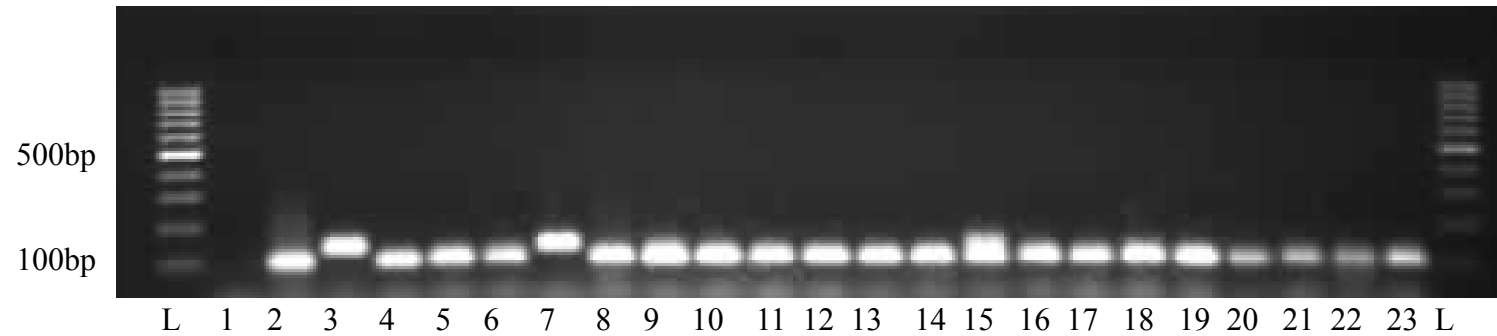


Figure 5.2. *Ggt* specific PCR analysis of 20 new *Ggt* isolates (BC21-BC40) obtained from root isolations of soil bioassay plants using primers Ggtfwd, GgtArev and GgtBrev2 to identify A and B *Ggt* subpopulations. Lane L, 100 bp DNA ladder (Promega); lane 1, no DNA; lane 2, positive control, *Ggt* A type isolate (92.15.4A), band size 93 bp; lane 3, positive control, *Ggt* B type isolate (99S9.4B), band size 132 bp; lanes 4-6 and 8-23, *Ggt* A type isolates BC21-BC23 and BC25-BC40, respectively; lane 7, *Ggt* B type isolate BC24.

5.3.2. Isolate characterisation by sensitivity to the fungicide silthiofam *in vitro*

Isolates from the new culture collection were then classified according to sensitivity to the fungicide silthiofam. Isolates were classified as resistant to silthiofam if the mean colony diameter on silthiofam amended PDA plates was at least 90% of that on unamended agar. According to this classification 30% of the isolates were characterised as resistant to silthiofam (Table 5.2). Variation in colony diameter between replicates was low (data not shown), and within the isolates classified as sensitive to silthiofam a wide range of sensitivities was apparent (Table 5.2). Seventeen of the thirty isolates classified as sensitive were almost completely inhibited by silthiofam with colony diameters less than 10% of that on unamended agar plates. Isolates with an intermediate level of sensitivity were also found with colony diameters up to 88% of that on control unamended plates.

Table 5.2. Sensitivity of isolates to the fungicide silthiofam as tested *in vitro* on silthiofam amended PDA plates.

Isolate	Mean % colony diameter on silthiofam amended plates/unamended plates ¹	Sensitive/Resistant classification ²
BC01	82.5	S
BC02	13.4	S
BC03	2.4	S
BC04	103.0	R
BC05	110.1	R
BC06	31.8	S
BC07	2.8	S
BC08	100.0	R
BC09	10.2	S
BC10	0.0	S
BC11	87.1	S
BC12	10.2	S
BC13	8.3	S
BC14	13.2	S
BC15	0.0	S
BC16	103.6	R
BC17	4.6	S
BC18	77.5	S
BC19	98.1	R
BC20	7.0	S
BC21	0.0	S
BC22	0.0	S
BC23	90.5	R
BC24	33.0	S

Table 5.2. Continued

Isolate	Mean % colony diameter on silthiofam amended plates/unamended plates ¹	Sensitive/Resistant classification ²
BC25	5.7	S
BC26	97.4	R
BC27	88.2	S
BC28	110.4	R
BC29	3.8	S
BC30	94.6	R
BC31	4.8	S
BC32	104.8	R
BC33	0.0	S
BC34	99.1	R
BC35	96.1	R
BC36	0.8	S
BC37	0.0	S
BC38	27.4	S
BC39	6.5	S
BC40	1.7	S

¹ increase in colony diameter of replicates on silthiofam amended and unamended PDA measured after 4 days at 21°C.

² isolates classified as resistant if mean colony diameter of the replicates on silthiofam amended PDA was at least 90% of that on control plates.

5.3.3. Relationship between genetic sub-population type and silthiofam sensitivity

No clear relationship was apparent between the genetic differentiation of isolates into A and B type and their sensitivity to silthiofam *in vitro*. The majority of isolates were A-type and sensitive to silthiofam (27/40, Table 5.3).

Table 5.3. Number of *Ggt* isolates from Bones Close culture collection that were classified as A or B type according to ITS2 diversity and sensitive or resistant to the fungicide silthiofam in *in vitro* fungicide sensitivity tests.

	A type	B type
Sensitive	27	1
Resistant	9	3

5.3.4. Isolate pathogenicity on wheat

A subset of 10 silthiofam resistant and 10 silthiofam sensitive isolates were selected for pathogenicity testing. The level of pathogenicity was measured as the percentage of wheat roots infected with take-all after 5 weeks. All twenty isolates were pathogenic on untreated wheat seeds with the percentage of roots infected ranging from 46-91% (Figure 5.3; Table 5.4). No very low pathogenicity isolates were found. This is to be expected as the isolates had originally been recovered from infected wheat roots and not directly from the soil.

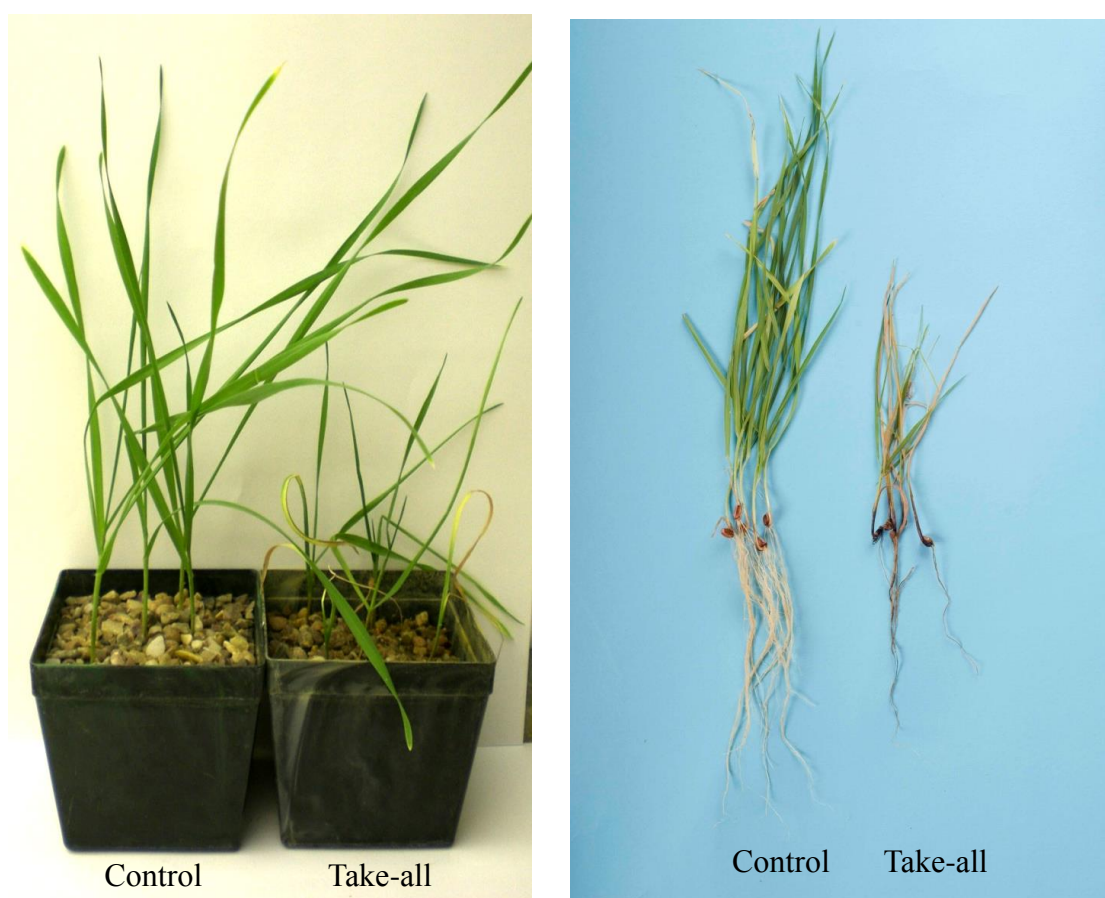


Figure 5.3. Pathogenicity test on wheat seedlings; left: control plant, mock-inoculated, right: *Ggt* infected seedling with yellowing leaves and stunted growth. Both panels were taken 5 weeks post inoculation.

The twenty isolates in the pathogenicity test were also tested for their sensitivity to silthiofam on plants grown from silthiofam treated wheat seed. All 10 isolates previously classified as sensitive on silthiofam amended PDA plates were sensitive on plants grown from silthiofam treated seed (Table 5.4). However four isolates (BC04, BC05, BC16 and BC32) previously identified as resistant to silthiofam *in vitro* were

fully sensitive on plants grown from treated seed (Table 5.4). These four isolates were tested again *in vitro* and were still found to be resistant.

Table 5.4. Pathogenicity of *Ggt* isolates with silthiofam treated wheat seed and untreated seed.

Isolate	Silthiofam sensitivity classification <i>in vitro</i> ¹	Mean percentage roots infected	
		Silthiofam treated seed	Untreated seed
BC02	sensitive	2.0	77.6
BC03	sensitive	0.0	81.3
BC06	sensitive	0.0	77.9
BC09	sensitive	0.0	90.8
BC10	sensitive	0.0	50.0
BC12	sensitive	0.0	87.3
BC13	sensitive	0.0	46.2
BC14	sensitive	0.0	83.2
BC15	sensitive	0.0	50.5
BC17	sensitive	0.0	70.5
BC04 ²	resistant	0.0	88.2
BC05 ²	resistant	0.0	74.2
BC16 ²	resistant	0.0	83.9
BC19	resistant	90.3	79.1
BC23	resistant	61.1	57.1
BC26	resistant	76.0	68.7
BC28	resistant	79.9	58.9
BC30	resistant	68.6	84.2
BC32 ²	resistant	0.0	74.5
BC34	resistant	78.5	70.1

¹ silthiofam sensitivity as tested on silthiofam amended PDA plates.

² silthiofam sensitive *Ggt* isolates previously classified as resistant to silthiofam in an *in vitro* fungicide sensitivity test which are fully sensitive on wheat plants grown from silthiofam treated seed.

5.4. Discussion

Forty *Ggt* isolates were successfully recovered by *Ggt* root isolations from infected soil bioassay plants. Isolates were characterised into two main genetic sub-populations according to ITS2 diversity using a *Ggt* specific PCR tool and also into two groupings based on sensitivity to the fungicide silthiofam. Mixtures of isolates, representative of field populations, could then be selected for screening wheat varieties for susceptibility to take-all (in Chapters 6 and 7).

Only a very small number of B-type isolates were recovered, with the majority of isolates (36 out of 40) being A type based on ITS diversity. This is similar to the study by Freeman et al. (2005) where the majority of isolates were also A type (116 out of 144 isolates).

Thirty percent of the new *Ggt* isolates were insensitive/resistant to silthiofam (>90% colony diameter on silthiofam PDA plates compared with unamended plates) despite being isolated from a field where silthiofam had not been used. Previous studies have shown that *Ggt* isolates that are insensitive to silthiofam occur naturally even in areas where populations have had no contact with silthiofam. For example, Freeman et al. (2005) reported that in six different field populations of isolates the percentage of insensitive isolates ranged from 10-30%. In the PhD study isolates classified as sensitive showed a range of sensitivities to silthiofam with colony diameters up to 88% of that on control unamended plates. Different 'naïve' *Ggt* isolates from the field have been reported to show large differences (10,000 fold) in the concentration of silthiofam required to inhibit growth (Joseph-Horne et al., 2000), suggesting that naturally in field populations a range of sensitivities are present. Freeman et al. (2005) also describe *Ggt* isolates with an intermediate level of sensitivity to silthiofam *in vitro*, but reports that the colony diameters of these isolates were only up to 50% of that on unamended control plates. Freeman et al. (2005) also reported that isolates with an intermediate sensitivity *in vitro* on silthiofam amended PDA plates were fully sensitive on plants that had been grown from silthiofam treated wheat seed. This was also true in the PhD study. However, in the PhD study four of the isolates that were classified as resistant to silthiofam *in vitro* (>90% colony diameter on unamended plates), were fully sensitive on silthiofam treated wheat seed in the pathogenicity test. The switch in silthiofam sensitivity between *in vitro* on PDA and on plants grown from silthiofam treated seed could potentially be due to differences in the activity of silthiofam in PDA plates and

treated wheat seed. Alternatively, the actual response of the *Ggt* isolates in PDA or in the soil or at the soil-wheat root interface could be different.

Silthiofam is specific to *Ggt* and is thought to inhibit ATP transport from mitochondria (Joseph-Horne et al., 2000). However, the exact target site that silthiofam acts upon is not known. Freeman *et al.* (2005) reported that there was a strong correlation between isolates that were sensitive to silthiofam *in vitro* and B-type based on molecular classification. Freeman et al. (2005) suggested that the polymorphism in ITS2 responsible for the A- and B-type genotypes could be linked to the actual causative mutation at the target site which is responsible for altering the sensitivity of isolates to silthiofam. In contrast in the PhD study there was no obvious correlation between sensitivity to silthiofam *in vitro* and classification as A or B type. However, the results from this work are not conclusive as only a relatively small number of isolates (40 isolates) from a single field (Bones Close) were tested in this study. In addition only 4 B-type isolates in total were characterised from the Bones Close culture collection. However, the use of the *Ggt* specific PCR assay as a method to assess the occurrence of insensitivity to silthiofam in field populations is not supported by the characterisation of field isolates from Bones Close.

For future use (in seedling pot test assays- Chapters 6 & 7) isolates were maintained on unamended PDA at 4°C or in long term water storage at room temperature as described in Chapter 2: General Materials and Methods.

CHAPTER 6: EVALUATION OF ELITE WINTER WHEAT VARIETIES FOR RESISTANCE TO TAKE-ALL

6.1. Introduction

As discussed in Chapter 1: General Introduction, there has been considerable effort by researchers to identify wheat varieties resistant to take-all. In the literature sometimes relatively large differences in the susceptibility of wheat varieties to take-all have been reported, but these differences have not been reliable or confirmed in further studies. Inconsistencies may partly be due to difficulties in assessing take-all disease and /or the masking of resistance due to disease patchiness and environmental interactions.

Before the start of the PhD project, a small number of hexaploid wheat varieties were assessed for resistance to take-all and surprisingly statistically significant differences were found in the severity of disease in a limited number of pot and field tests (Richard Gutteridge, unpublished data). At the same time consistent differences in the ability of wheat varieties to encourage the build-up of the take-all fungus during a first wheat crop were being demonstrated, suggesting that there were useful genetic interactions between hexaploid wheat varieties and the take-all fungus (Chapter 3: Field evaluation of the take-all inoculum build-up trait)(McMillan et al., 2011). In the light of this information the susceptibility of wheat varieties to take-all was re-examined in this study using a series of three consecutive field trials and a seedling pot test method. Fifty current UK HGCA Recommended List, previously recommended or candidate wheat varieties were chosen to be evaluated for a thorough study of the susceptibility of modern wheat varieties to take-all.

Studying take-all in the field is a challenge due to the patchy distribution of disease in the soil. It is hard to detect treatments effects when there is usually a large degree of background variation in field trials (Bateman & Hornby, 1995). Artificial inoculum has been applied in field trials to try to decrease the variability across trial sites in the initial distribution of inoculum (Bateman & Hornby, 1999). Cotterill and Sivasithamparam (1989a) found that variability in the distribution of disease was significantly reduced in a second wheat crop after addition of artificial inoculum during the first wheat crop. Hornby and Bateman (1990) used oat grains colonised by *Ggt* to infest artificially the soil in a spring wheat. This also tended to create quite uniform and severe disease. However, applying artificial inoculum did not produce a uniform distribution of disease in further experiments with winter wheat (Bateman & Hornby, 1995). In a more recent

study Gutteridge et al. (2003) reported that there were no marked differences between artificial and natural inoculum sources when investigating the effect of take-all on grain yield, suggesting no distinct benefit of using artificial inoculum. In field experiments in Australia artificial inoculum caused earlier disease, yellowing and stunting of plants compared with naturally infected plants (Jensen & Joergensen, 1973). Artificial inoculum cannot therefore be assumed to reliably simulate natural take-all epidemics. In this PhD study the susceptibility of wheat varieties to take-all was carried out using third wheat field trials, naturally infested with take-all, for an expected good natural disease pressure. Naturally infested sites were chosen as there is no strong evidence that artificially applied inoculum acts in the same way as natural inoculum, or that it reliably reduces variability across trial sites. To partition effectively the natural background variation an alpha field trial design was used (as in Chapter 3 for the first wheat elite winter wheat variety and TAB field trials). In this design the 45 varieties within each of the four large replicate blocks were further grouped into smaller incomplete sub-blocks. Variety means are then formed from weighted sums of the variety values from the sub-blocks.

Take-all disease was assessed in the spring as the percentage of plants with take-all and the number of seminal and crown roots infected. In the summer take-all disease was assessed using a take-all index on plant samples collected during grain filling. Full details of the calculation of the take-all index are given in Chapter 2: General Materials and Methods. The take-all index is based on the extent of root blackening of whole plant root systems and has previously been described as a measure of take-all intensity (Bateman et al., 2008). The take-all index has been used in a number of studies at Rothamsted to evaluate the control of take-all by fungicidal seed treatments (Bateman et al., 2004, Bateman et al., 2006, Bateman et al., 2008). Take-all disease measurement during grain filling is generally considered to correlate best with yield (Hornby et al., 1998). Although often the effect of disease is confounded by other factors such as drought stress and nutrient deficiencies, which can exacerbate the problems of take-all. The ability of other plants to compensate for severely infected plants also makes it hard to determine losses in yield. However, in a previous study the take-all index has been significantly related to yields (Gutteridge et al., 2006). Bateman et al. (2004) also report that when take-all seed treatments decreased take-all, grain yields typically increased. Plot yields were recorded in the three third wheat trials in the PhD study. However, wheat varieties have inherently different yields, regardless of whether take-all is present (HGCA Recommended Lists, www.hgca.com). The same wheat varieties were sown in

first wheat field trials on the Rothamsted farm in the same years (reported in Chapter 3: Field evaluation of the TAB trait). The percentage disease loss between the varieties sown as a first wheat without take-all and in the third wheat trials could then be calculated to identify varieties that yield best in a take-all situation, and so may show some tolerance to take-all infection.

A take-all patch score was also employed in the study to assess the above ground symptoms of take-all. The above ground appearance of the crop as a measure of take-all has previously been used in the 1980s and 1990s in UK winter wheat disease surveys (Polley & Thomas, 1991, Hornby et al., 1998). In these studies categories of 0-4 were used for field scale assessments: 0 = no symptoms of take-all, 1 = a scatter of prematurely ripened plants, 2 = some small patches of stunted prematurely ripened plants, 3 = many small, or some large patches, 4 = many large areas with above ground symptoms. Since the late 1990s researchers at Rothamsted have assessed plots for the above ground symptoms of take-all by estimating the percentage of each plot area showing prematurely ripened stunted plants caused by take-all (Bateman & Hornby, 1999, Gutteridge et al., 2006, Bateman et al., 2008). This was the method chosen in this study.

A more detailed epidemiology study was carried out on a subset of wheat varieties during the growing season. In 2009 and 2010 this formed part of HGCA bursary projects with students James Bruce (2009) and Nicola Phillips (2010). The aim was to look at how the dynamics of disease differed between varieties. The rate of disease development could be an important influence on yield loss.

Pot tests with artificial inoculum were chosen to assess the susceptibility of varieties to take-all as seedlings and to compare this to field performance. The use of a reliable pot screening method could speed up the selection of genotypes for more detailed field screening in future work. Laboratory studies using pot tests with artificial *Ggt* inoculum have previously been used to assess the pathogenicity of *Ggt* isolates on different cereal hosts (Hollins et al., 1986, Hollins & Scott, 1990), and for screening wheat varieties for resistance to take-all (Nilsson, 1969, Nilsson, 1973, Scott, 1981, Penrose, 1985, Penrose, 1992, Eastwood et al., 1994, Penrose & Neate, 1994). These tests have most commonly used sand, vermiculite or compost as the growth medium for wheat plants. In the literature it has been hard to compare studies due to the different methods employed to establish and measure disease. In addition, some studies have used very high levels of inoculum and this could mask differences in susceptibility to take-all (Scott, 1981).

A pot test method was established at Rothamsted to test the pathogenicity of take-all isolates to wheat and rye seedlings (Gutteridge et al., 1993). This method originally used a silver sand-coarse grit growing medium in the pots and either PDA plugs of *Ggt* isolates or sand/maizemeal cultures as the source of artificial inoculum. A modified version of this pot test used naturally infested soil to test the effect of the fungicide Amistar® (Syngenta, UK) on take-all disease (Jenkyn & Gutteridge, 2002). Further modification to use take-all free soil with addition of artificial inoculum has since been developed at Rothamsted to test the efficacy of various fungicides (Richard Gutteridge, personal communication, 2009). The aim has been to obtain a uniform level of infection by which to compare treatments and tests. Field soil is collected from take-all free fields (fields not sown with cereals) and sand/maizemeal *Ggt* cultures are used as the source of artificial inoculum. Using natural unsterilised field soil should allow a better prediction of field performance. A soil calibration test is always carried out first to find out the appropriate dilution of sand/maizemeal culture to add to the soil. The aim has been to achieve 50% roots infected on the standard wheat variety Hereward, to ensure moderate levels of infection but not so much as to potentially mask the effect of treatments on take-all disease. Typically a mixture of approx. ten *Ggt* isolates in sand/maizemeal culture is used in these tests, as a representation of field populations of the fungus. In Chapter 5 a new *Ggt* isolate collection was characterised and 40 isolates classified as sensitive or resistant to the fungicide silthiofam and A or B type based on a molecular PCR assay. Three mixtures of isolates were then used in the pot tests reported in this chapter to test the susceptibility of wheat varieties to the different subpopulations of *Ggt* isolates. Hollins & Scott (1990) have previously shown that individual *Ggt* isolates varied widely in their pathogenicity to the species rye and also differed slightly in their pathogenicity to wheat. In the PhD study a mixture of five silthiofam resistant isolates and a second mixture of five silthiofam sensitive isolates were first selected for testing. All of these isolates were A-type. Only a very small number of B-type isolates (4/40) were recovered, and these were all sensitive to the fungicide silthiofam when tested in a pathogenicity test with silthiofam treated wheat seed. These four isolates were selected as the third mixture. B-type isolates that were silthiofam resistant could not be tested. In the pot test disease is assessed by observing the number of roots with take-all lesions. The total number of roots in each pot is also counted so that the amount of take-all disease is expressed as a percentage of the total root system. Scott (1981) points out that the amount of pathogen growth could be masked by differences in rooting ability between varieties when the proportion of a root system infected is recorded. The

number of infected roots per plant and total number of roots were therefore also reported in the PhD study so that the influence of rooting ability could be considered in the assessment of disease.

6.2. Materials and Methods

6.2.1. Elite winter wheat field trials

Three winter wheat field trials, in the harvest years of 2009, 2010 and 2011 were set up to evaluate the take-all susceptibility of modern elite winter wheat varieties (Table 6.1). The standard procedures for evaluating winter wheat germplasm for resistance to take-all in the field are described in Chapter 2: General Materials and Methods. All three trials were sown as third wheat crops after two previous winter wheat crops. In 2009 and 2010 the trials consisted of four replicates of 45 winter wheat varieties with additional incomplete sub-blocking within replicates. This basic design with the additional sub-blocking within whole blocks is an alpha design. The arrangements were all generated by Rodger White using CycDesigN (VSN International Limited, Hemel Hempstead, UK). There were three replacement varieties in 2010 due to limited seed availability of the original varieties (Table 6.2). The same 45 elite wheat varieties in 2009 and 2010 were also evaluated for take-all inoculum build-up in first wheat field trials (Chapter 3: Evaluation of the take-all inoculum build-up trait). In 2011 the number of varieties screened for susceptibility to take-all was reduced to 10 varieties based on the results from 2009 and 2010, and also included two new varieties Kingdom (Syngenta Seeds) and KWS Stirling (KWS UK). Due to the smaller size of the 2011 trial no sub-blocking within whole blocks was used. Field trial plans are given in Appendix 6.1. Plots were sown in the autumn at a seed rate of 350 seeds/m² and yields were taken from each plot the following summer by the Rothamsted farm.

Plant samples were taken in both the spring and summer from all plots for take-all disease assessments (see Chapter 2: General Materials and Methods). In the spring whole plant samples were dug from five 15 cm lengths of row and in the summer samples were taken from ten 20 cm lengths of row (Table 6.4). In 2009 the extent of take-all patches was recorded per plot on the 9th July at GS 75. In 2010 and 2011 there were no clearly visible take-all patches in the trials so the extent of take-all patches was not recorded. Data was statistically analysed by Rodger White. Percentage disease data was always transformed using the logit transformation, to ensure equal variance. In 2009 and 2010 transformed data was analysed using the REML procedure in Genstat to

incorporate the sub-blocking structure. In 2011 the disease variables were analysed by analysis of variance. A combined year analysis on the effect of variety on take-all in the spring and summer of all three years was carried out using REML.

Epidemiology studies on take-all disease development were carried out on six selected varieties in 2009 (Cordiale, Einstein, Hereward, Robigus, Solstice and Xi19). In 2010 eight varieties were selected (the same six varieties as 2009 plus Avalon and Cadenza). In 2011 the epidemiology studies were carried out on two of the ten wheat varieties in the trial (Hereford and Hereward). Varieties selected for epidemiology studies are shown in Table 6.3. The original six varieties in 2009 were primarily selected based on a limited number of previous pot and field tests. In 2010 Avalon and Cadenza were added to look in detail at their third wheat performance compared with their consistent contrasting ability to build-up inoculum during a first wheat crop (for the evaluation of take-all inoculum build-up during the first wheat crop see Chapter 3). In 2011 Hereford was chosen for the epidemiology study due to its good performance in the 2009 field trial, with the lowest take-all index out of the 45 varieties tested in that year. Hereward was included as a fully susceptible control. In 2009 and 2010 the epidemiology studies were part of HGCA funded summer bursary projects with students James Bruce (2009) and Nicola Phillips (2010).

Plant samples (5 x 15 cm lengths of row per plot) for the epidemiology studies were taken at monthly intervals from March or April through to the summer sampling point in July when plant samples (10 x 20 cm lengths of row per plot) were taken from all plots (Table 6.4). Samples were washed free from soil and examined for take-all lesions in a white dish under water. The total number of plants and the number of take-all infected plants, seminal and crown roots were recorded at each sampling point. Subsamples of 10 plants per plot were chosen at random for root counts of the mean number of seminal and crown roots per plant. A cross-season analysis was carried out using a repeated measurements ANOVA in Genstat.

Table 6.1. Details of the field experiments used to evaluate the susceptibility to take-all of elite winter wheat varieties.

Harvest year		Previous cropping history				
(Rothamsted field trial code)	Rothamsted Field	Preceding year	2 years previous	Sowing date	Plot size (m)	Date harvested
2009 (09/R/WW/917)	Stackyard	Winter wheat	Winter wheat	09/10/2008	10 x 2	12-13/08/2009
2010 (10/R/WW/1031)	West Barnfield	Winter wheat	Winter wheat	19/10/2009	9 x 1.8	17/08/2010
2011 (11/R/WW/1114)	Claycroft	Winter wheat	Winter wheat	14/10/2010	10 x 1.8	24/08/2011

Table 6.2. Elite winter wheat varieties used in field experiments 09/R/WW/917, 10/R/WW/1031 and 11/R/WW/1114.

Code	Variety	No of years in trials	Nabim Group ¹	Date first Listed ²	Breeder ³	Parentage ⁴
Al	Alchemy	2	4	2006	Nick	Claire x (Consort x Woodstock)
Av	Avalon	2	1	1980	PBI	TJB 30/148 x TL 365A/34
Bn	Bantam	2	4	NR (2008)	Nick	Xi19 x NSL WW35
Bt	Battalion	2	2	2007	RAGT	98ST08 x Aardvark
Br	Brompton	2	4	2005	Els	CWW 92.1 x Caxton
Ca	Cadenza ⁵	2	2	1994	CPB	Axona x Tonic
Cs	Cassius	2	4	2009	Nick	Claire x (NSL WW24 x Wizard)
Cl	Claire	2	3	1999	Nick	Wasp x Flame
Cn	Conqueror	2	4	2010	KWS	Robigus x Equinox
Cr	Cordiale	2	2	2004	CPB	(Reaper x Cadenza) x Malacca
Du	Duxford	3	4	2008	NFC	Solstice x Scorpion 25
Ed	Edmunds	3	3	NR (2009)	Nick	Deben x Napier
Ei	Einstein	2	2	2003	Nick	(NHC49 x UK Yield Bulk) x (Haven x Clarion)

Table 6.2. Continued

Code	Variety	No of years in trials	Nabim Group ¹	Date first Listed ²	Breeder ³	Parentage ⁴
Ga	Gallant	2	1	2009	Syn	(Malacca x Charger) x Xi19
Gl	Gladiator	2	4	2004	Mon	Falstaff x Shannon
Gw	Glasgow	1	4	2005	SU	(Ritmo x SUR 90-2666) x SUR 91-11658
Gr	Grafton	2	4	2009	KWS	Cordiale x CPBT W97
Hf	Hereford	2	4	NR (2007)	Sej	Solist x Deben
Hw	Hereward	3	1	1991	PBI	Norman 'sib' x Disponent
Hu	Humber	2	4	2007	CPB	Anglo x Krakatoa
Hy	Hyperion	2	4	2006	Nick	Aardvark x (Consort x Woodstock)
In	Invicta	3	3	2010	Nick	NSLWW48 x Robigus
Is	Istabraq	2	4	2004	Nick	Consort x Claire
Jb	JB Diego	2	4	2008	Breun	3351b x Stru2374
Ke	Ketchum	2	2	2009	Syn	Solstice x Xi19
Kg	Kingdom	1	2	2010	Syn	Cordiale x Xi19
Ki	Kipling	3	4	NR (2006)	Depr	Hunter x 9205-4
Kw	KWS Sterling	1	2	2010	KWS	(Quest x Wizard) x Cordiale
Le	Lear	3	4	NR (2008)	Nick	Robigus x Nijinsky
Ml	Malacca	2	1	1999	CPB	Riband x (Rendevouz) x Apostle
Mw	Maris Widgeon	1	1	1964	PBI	Holdfast x Cappelle-Desprez
Mr	Marksman	2	2	2008	RAGT	98ST08 x Aardvark
Ms	Mascot	2	1	2006	RAGT	Reaper x Rialto
Mn	Monty	1	4	NR (2007)	Syn	Robigus x NFC10035
Oa	Oakley	2	4	2007	CPB	(Aardvark 'sib' x Robigus) x Access
Pn	Panorama	2	2	2009	Nick	(Xi19 x Solstice) x Solstice

Table 6.2. Continued

Code	Variety	No of years in trials	Nabim Group ¹	Date first Listed ²	Breeder ³	Parentage ⁴
Pa	Paragon ⁶	1	1	1999	PBI	CSW 1724/19/6/68 x (Axona x Tonic)
Qp	Qplus	2	2	2009	Nick	Solstice x Robigus
Ri	Riband	2	4	1989	PBI	Norman x (Maris Huntsman x TW161)
Ro	Robigus	2	3	2003	CPB	Z836 x 1366
Sc	Scout	2	3	2009	Sen	Z435 x Deben
Se	Sherborne	2	4	NR (2007)	KWS	Aardvark sib x Biscay
Sh	Shogun	2	4	NR (2008)	RAGT	Mallet x Whistler
Si	Soissons	2	2	1995	Depr	Jena x HN 35
So	Solstice	3	1	2002	Adv	Vivant x Rialto
Vi	Viscount	2	4	2009	KWS	Robigus x Canterbury
Wa	Walpole	1	2	NR (2008)	Nick	Xi19 x Solstice
We	Welford	2	4	2004	Els	CWW 92/1 x FD92054
Xi	Xi19	2	1	2002	Adv	(Cadenza x Rialto) x Cadenza
Ze	Zebedee	2	3	2007	Nick	Claire x Nelson

¹ Nabim groups; Group 1 = quality breadmaking wheats, Group 2 = breadmaking potential wheats, Group 3 = biscuit wheats, Group 4 = feed wheats.

² Date first recommended. NR = Not recommended (first candidate year).

³ Original breeder in year first listed. Adv, Advanta Seeds UK; Breun, Saatzucht Josef Breun, Germany; CPB, CPB Twyford; Depr, Maison Florimond Desprez, France; Els, Elsoms Seeds; KWS, KWS UK; Mon, Monsanto; NFC, New Farm Crops; Nick, Nickersons; PBI, Plant Breeding Institute; RAGT, RAGT Seeds; Sej, Sejet, Denmark; Sen, Senova; SU, Saaten Union UK; Syn, Syngenta Seeds.

⁴ Parentage information obtained from breeder websites, archive HGCA Recommended Lists and NIAB association pocket guides to varieties of cereals, oilseeds and pulses.

⁵ Cadenza = facultative spring wheat

⁶ Paragon = spring wheat

Table 6.3. Varieties selected for epidemiology studies in field trials 09/R/WW/917, 10/R/WW/1031 and 11/R/WW/1114.

Variety	No. of years in epidemiology studies	Biological feature of interest
Avalon	1 (2010)	high TAB ¹
Cadenza	1 (2010)	low TAB ¹
Cordiale	2 (2009 & 2010)	good second wheat yield ²
Einstein	2 (2009&2010)	high susceptibility to take-all in a limited number of field trials ³
Hereford	1 (2011)	lowest take-all index in 2009 PhD study field trial (09/R/WW/917)
Hereward	3 (2009, 2010 & 2011)	highly susceptible variety used as control in take-all field trials at Rothamsted
Robigus	2 (2009 & 2010)	poor second wheat yield ²
Solstice	2 (2009 & 2010)	low susceptibility to take-all in seedling pot test ³
Xi19	2 (2009 & 2010)	low susceptibility to take-all in seedling pot test ³

¹ Take-all inoculum build-up (TAB) during a first wheat crop, varieties classified based on performance in WGIN field trials (McMillan et al., 2011).

² HGCA Recommended List® for Winter Wheat 2009/2010 yield data.

³ Unpublished data (Richard Gutteridge).

Table 6.4. Sampling details of the 3rd wheat elite winter wheat variety and susceptibility to take-all field trials 2009-2011.

Harvest year (field trial code) Varieties sampled	Sampling method/ additional measurements	Units ¹ per plot	Month	Date sampled	Growth stage ² (GS)
2009 (09/R/WW/917)					
All varieties	Plant samples	5 x 15 cm	April	20-21/04/2009	22/23
Epidemiology 6 varieties	Plant samples	5 x 15 cm	May	22/05/2009	37
	Plant samples	5 x 15 cm	June	17/06/2009	60
All varieties	Plant samples	10 x 20 cm	July	06-08/07/2009	75
	Take-all patch score	% area	July	09/07/2009	75
2010 (10/R/WW/1031)					
All varieties	Plant samples	5 x 15 cm	April	21/04/2010	30
Epidemiology 8 varieties	Plant samples	5 x 15 cm	May	17/05/2010	37
	Plant samples	5 x 15 cm	June	17/06/2010	61
All varieties	Plant samples	10 x 20 cm	July	12-13/07/2010	75
2011 (11/R/WW/1114)					
Epidemiology 2 varieties	Plant samples	5 x 15 cm	March	17/03/2011	14
All varieties	Plant samples	5 x 15 cm	April	18/04/2011	23/24
Epidemiology 2 varieties	Plant samples	5 x 15 cm	May	16/05/2011	37
	Plant samples	5 x 15 cm	June	20/06/2011	71
All varieties	Plant samples	10 x 20 cm	July	18/07/2011	81

¹ Plant sampling units in lengths of row per plot (cm).

² Zadoks decimal code for cereals.

6.2.2. Elite winter wheat pot tests

The standard procedures for preparing inoculum, preparing soil, carrying out an inoculum-soil calibration and setting up the pot test are described in Chapter 2: General Materials and Methods.

In July 2009 the 45 elite winter wheat varieties in the 2009 field trial were evaluated for their susceptibility to take-all at the seedling stage in a five week pot test. Soil was collected from the Rothamsted field 'Great Field IV', taken from a 2nd year fallow before any cultivation in August 2008. A mixture of five isolates classified as resistant (R) to the fungicide silthiofam (BC19, BC23, BC26, BC28 and BC34) and a separate mixture of five isolates classified as sensitive (S) to silthiofam (BC02, BC03, BC10, BC15 and BC17) were selected for use in the test based on their characterisation in fungicide classification tests described in Chapter 5: Characterisation of a new *Ggt* isolate collection. All ten isolates were A type based on molecular characterisation. Dilutions of 1:150 and 1:200 of the artificial sand/maizemeal *Ggt* inoculum with silver sand were used for the resistant and sensitive isolate mixtures respectively, with 50 g of this dilute inoculum being added to 250 g of the soil. Five replicates were set up per wheat variety with the silthiofam resistant mix of isolates and five replicates with the silthiofam sensitive mix of isolates. Control pots of 250 g soil and 50 g silver sand without take-all were set up with the control susceptible wheat variety Hereward (10 replicates).

In May 2011 the same 45 elite wheat varieties were evaluated for their susceptibility to take-all at the seedling stage in a five week pot test using B type *Ggt* isolates based on molecular classification. Soil was collected in July 2009 from a 3rd year fallow site in the Rothamsted field 'Great Field IV'. A mixture of the four B type isolates (BC04, BC05, BC16 and BC24) from the new *Ggt* isolate collection was used. All four B type isolates were fully sensitive to the fungicide silthiofam on wheat plants grown from silthiofam treated seed. Due to a low level of infection on Hereward plants in previous soil calibrations the protocol was modified to mix 150 g soil with 100 g damp sand before adding 50 g of a 1:50 dilution of artificial sand/maizemeal *Ggt* inoculum with silver sand. Five replicates were set up per wheat variety with 5 control pots of 250 g soil and 50 g silver sand without take-all with the control wheat variety Hereward. Pots were placed in a completely randomised design in the controlled environment room (16 hour day, 70% RH, 15°C day/10°C night, twice weekly watering) for five weeks before take-all disease assessment.

Disease variable data were analysed using analysis of variance in Genstat. Spearman's rank correlation was used to explore the relationships between different variates and between the seedling pot test and performance in field trials.

6.3. Results

6.3.1. Response of winter wheat varieties to take-all under field conditions

2009

In 2009 the incidence of take-all (% plants infected) in the spring was high for all 45 varieties tested, ranging from 62.2% to 95.8% plants infected. The total number of take-all infected roots per plant ranged from 1.24 to 2.45. There were no significant varietal differences for either disease parameter (Logit % plants infected: $P = 0.246$; take-all infected roots per plant: $P = 0.097$). The total number of roots per plant can be further examined by looking at the number of seminal and crown roots infected per plant. There were close to significant varietal differences in the number of infected seminal roots per plant ($P = 0.067$) but not in the number of crown roots infected per plant ($P = 0.155$).

In summer 2009 significant varietal differences were now detected in the percentage of plants infected ($P = 0.002$) and take-all index ($P < 0.001$) of the winter wheat varieties tested (Table 6.5). Over 95% of plants were infected for all varieties, indicating a high take-all disease pressure across the field trial. The take-all index combines incidence and severity of take-all by taking into account the percentage of plants infected into 5 different categories based on severity from slight to severe. The mean take-all index (0-100) across the trial was 74.07, and variety means ranged from 56.48 to 83.86. The winter wheat variety with the lowest take-all index was Hereford (Breeder: Sejet, Denmark). The variety with the 2nd lowest take-all index was Cassius (Breeder: Nickersons, UK), although its take-all index was nearly 10 points higher than Hereford. Hereford therefore stood out as a variety potentially displaying some partial resistance to take-all.

There were no strong significant correlations between the spring and summer take-all disease parameters using Spearman's rank correlation analysis (data not shown).

Table 6.5. Take-all disease in the spring and summer of the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).

2009	Spring				Summer				
	Variety ¹	Logit % plants infected (BT means)	No. infected roots/plant	No. infected seminal roots/plant	No. infected crown roots/plant	TAI (0-100)	Logit % plants infected (BT means)	TA patch (% area)	Yield (tonnes/ha)
	Alchemy	1.02 (88.5)	2.11	1.76	0.35	66.75	2.37 (99.1)	-1.75 (14.9)	7.68
	Avalon	0.79 (82.8)	1.51	1.34	0.16	71.61	2.48 (99.3)	-0.44 (39.1)	7.02
	Bantam	1.20 (91.7)	2.07	1.53	0.53	69.01	2.06 (98.4)	-1.43 (19.2)	7.82
	Battalion	0.94 (86.7)	1.74	1.44	0.30	78.62	2.47 (99.3)	0.25 (56.2)	7.74
	Brompton	1.04 (89.0)	1.83	1.52	0.31	83.65	2.43 (99.2)	-1.09 (25.1)	7.70
	Cadenza	0.74 (81.6)	2.00	1.71	0.29	73.10	2.46 (99.3)	-0.51 (37.6)	7.06
	Cassius	0.75 (81.6)	1.59	1.31	0.27	65.81	2.20 (98.8)	-1.57 (17.2)	8.60
	Claire	0.74 (81.4)	1.80	1.49	0.30	79.41	2.53 (99.4)	-0.63 (34.8)	7.10
169	Conqueror	0.74 (81.4)	1.67	1.34	0.32	75.62	2.22 (98.8)	-0.68 (33.5)	7.89
	Cordiale	0.79 (83.0)	1.79	1.45	0.34	71.02	2.45 (99.3)	0.90 (71.0)	7.54
	Duxford	0.84 (84.4)	1.80	1.53	0.27	66.52	2.14 (98.6)	-1.91 (12.9)	7.50
	Edmunds	1.00 (88.1)	2.45	1.88	0.57	80.87	2.52 (99.4)	-0.77 (31.7)	7.85
	Einstein	1.17 (91.2)	1.35	1.19	0.17	74.96	2.50 (99.3)	-0.15 (46.2)	7.04
	Gallant	1.04 (88.9)	1.59	1.44	0.15	76.19	2.37 (99.1)	0.93 (71.7)	7.27
	Gladiator	0.62 (77.6)	1.24	1.12	0.12	80.41	2.42 (99.2)	0.06 (51.5)	7.71
	Grafton	0.87 (85.2)	1.49	1.27	0.21	69.98	2.26 (98.9)	1.00 (73.1)	7.57
	Hereford	0.84 (84.3)	1.39	1.16	0.23	56.48	1.64 (96.3)	-1.70 (15.5)	8.81
	Hereward	0.88 (85.4)	1.62	1.40	0.22	75.72	2.33 (99.1)	-1.21 (23.0)	6.86
	Humber	1.39 (94.1)	2.14	1.88	0.27	75.04	2.31 (99.0)	-0.54 (36.9)	7.45
	Hyperion	0.63 (77.8)	1.33	1.18	0.16	76.80	2.11 (98.5)	-0.78 (31.4)	8.09
	Invicta	0.71 (80.6)	2.01	1.56	0.44	66.41	2.44 (99.3)	-1.93 (12.6)	8.17
	Istabraq	0.90 (85.9)	1.57	1.40	0.18	68.46	2.14 (98.6)	-1.97 (12.2)	8.00

Table 6.5. Continued

2009	Spring				Summer			
Variety ¹	Logit % plants infected (BT means)	No. infected roots/plant	No. infected seminal roots/plant	No. infected crown roots/plant	TAI (0-100)	Logit % plants infected (BT means)	TA patch (% area)	Yield (tonnes/ha)
JB Diego	0.93 (86.6)	1.77	1.48	0.29	76.22	2.48 (99.3)	-1.03 (26.4)	8.23
Ketchum	0.65 (78.5)	1.63	1.34	0.29	77.95	2.35 (99.1)	-1.17 (23.7)	8.33
Kipling	0.84 (84.3)	1.53	1.37	0.16	80.88	2.20 (98.8)	-0.45 (38.9)	6.83
Lear	0.63 (77.9)	1.58	1.36	0.23	67.40	2.42 (99.2)	-1.75 (14.8)	8.58
Malacca	0.93 (86.6)	1.79	1.52	0.27	72.53	2.45 (99.3)	-0.68 (33.6)	7.64
Marksman	0.78 (82.7)	1.54	1.27	0.27	78.17	2.47 (99.3)	0.88 (70.7)	6.99
Mascot	1.56 (95.8)	1.73	1.58	0.14	80.41	2.34 (99.1)	-0.52 (37.2)	7.14
Monty	0.66 (78.9)	1.52	1.28	0.23	83.86	2.46 (99.3)	-0.69 (33.4)	7.81
Oakley	0.91 (86.1)	1.77	1.42	0.35	78.33	2.53 (99.4)	-0.96 (27.8)	8.39
Panorama	0.88 (85.4)	1.76	1.54	0.23	77.16	2.04 (98.4)	-1.28 (21.8)	7.72
Qplus	0.25 (62.2)	1.33	1.07	0.26	74.52	2.29 (99.0)	-0.91 (28.7)	7.71
Riband	0.90 (85.8)	1.45	1.08	0.37	77.54	2.47 (99.3)	-1.63 (16.3)	8.04
Robigus	0.80 (83.1)	1.62	1.40	0.23	70.99	2.33 (99.1)	-0.94 (28.2)	7.35
Scout	1.17 (91.2)	2.25	1.89	0.37	78.56	2.54 (99.4)	-0.78 (31.5)	6.97
Sherborne	0.75 (81.8)	2.03	1.58	0.43	70.50	2.35 (99.1)	-1.06 (25.8)	7.92
Shogun	0.98 (87.7)	1.68	1.40	0.29	67.57	2.14 (98.6)	-0.12 (46.9)	8.32
Soissons	0.72 (81.0)	1.48	1.23	0.24	78.14	2.37 (99.1)	-2.18 (10.2)	6.26
Solstice	0.74 (81.6)	1.51	1.35	0.16	67.92	1.95 (98.0)	-1.01 (26.7)	7.10
Viscount	0.59 (76.4)	1.38	1.22	0.17	71.26	2.37 (99.1)	-1.11 (24.8)	7.37
Walpole	0.77 (82.3)	2.03	1.65	0.38	71.35	2.31 (99.0)	-2.24 (9.7)	8.34
Welford	1.03 (88.7)	1.75	1.37	0.38	81.65	2.55 (99.4)	-0.87 (29.5)	7.65
Xi19	0.55 (74.9)	1.34	1.22	0.11	68.58	2.38 (99.1)	-0.97 (27.5)	6.97
Zebedee	1.01 (88.3)	1.94	1.65	0.28	79.18	2.37 (99.1)	-0.28 (43.1)	7.74

Table 6.5. Continued

2009	Spring				Summer			
	Logit % plants infected (BT means)	No. infected roots/plant	No. infected seminal roots/plant	No. infected crown roots/plant	TAI (0-100)	Logit % plants infected (BT means)	TA patch (% area)	Yield (tonnes/ha)
d.f.	44	44	44	44	44	44	44	44
SED Average	0.293	0.327	0.242	0.127	4.763	0.181	0.343	0.385
Wald statistic	51.68	60.25	63.02	56.25	128.33	88.22	514.00	184.69
F Probability	0.246	0.097	0.067	0.155	<0.001	0.002	<0.001	<0.001
Grand mean	0.86 (84.0)	1.70	1.43	0.27	74.07	2.33 (99.0)	-0.84 (32.1)	7.64

¹ **Bold** = varieties in epidemiology study.

At the beginning of July 2009 the above ground symptoms of take-all showing as prematurely ripened and stunted plants were visible throughout the field trial. The mean varietal take-all patch scores recorded based on these visible symptoms varied from 10% to 73% of the plot area affected (Table 6.5). While scoring in the field it was noticed that some varieties (for example Soissons) were harder to score for the extent of take-all patches due to the golden colour of leaves and ears. This above ground take-all patch score was weakly positively correlated with the take-all index of plant samples ($R_s = 0.39$, $P = 0.01$, $n = 45$; Figure 6.1). There were four obvious outlying varieties in this correlation analysis which had high mean take-all patch scores compared with the level of take-all root infection (indicated by a red circle in Figure 6.1). These varieties were Cordiale, Gallant, Grafton and Marksman. The winter wheat variety Hereford had the lowest take-all index of the varieties tested and also had one of the lowest mean take-all patch scores (red diamond symbol in Figure 6.1).

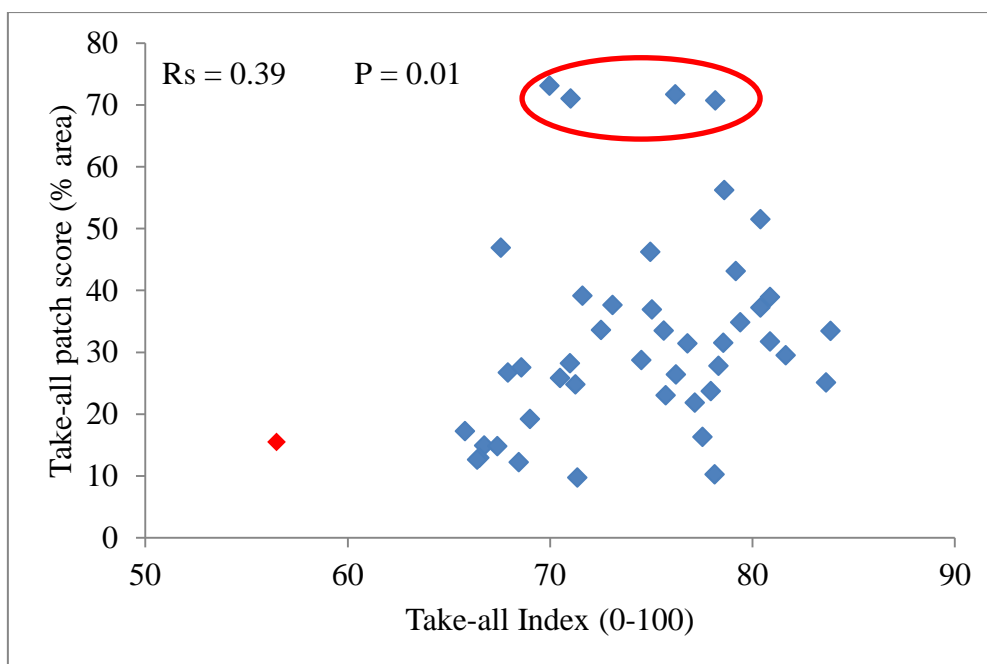


Figure 6.1. Correlation between the mean varietal take-all patch score and take-all index of plant samples in the 2009 third wheat field trial (09/R/WW/917). Red circle = varieties with a higher take-all patch score compared with the take-all index of plant samples. Red diamond = winter wheat variety Hereford, with the lowest take-all index out of the varieties tested and also a low take-all patch score.

Plot yields taken by the Rothamsted farm at harvest of the third wheat field trial revealed highly significant differences between varieties ($P = <0.001$; Table 6.5). Yields were also taken from the first wheat field trial (with negligible take-all root infection) of the same 45 varieties x 4 reps (09/R/WW/916; Chapter 3). The percentage yield loss of

the 45 wheat varieties grown at the third wheat high take-all disease pressure could then be calculated compared with their yields without take-all disease in the first wheat trial. Variety yields were on average 39.15% lower in the third wheat field trial compared with the first wheat field trial. The poor performance of wheat varieties grown at a high take-all disease pressure in the third wheat trial was clearly visible when compared with the same wheat varieties grown in the first wheat field trial (Figure 6.2). The winter wheat variety Hereford had the highest average 3rd wheat yield (Table 6.5).

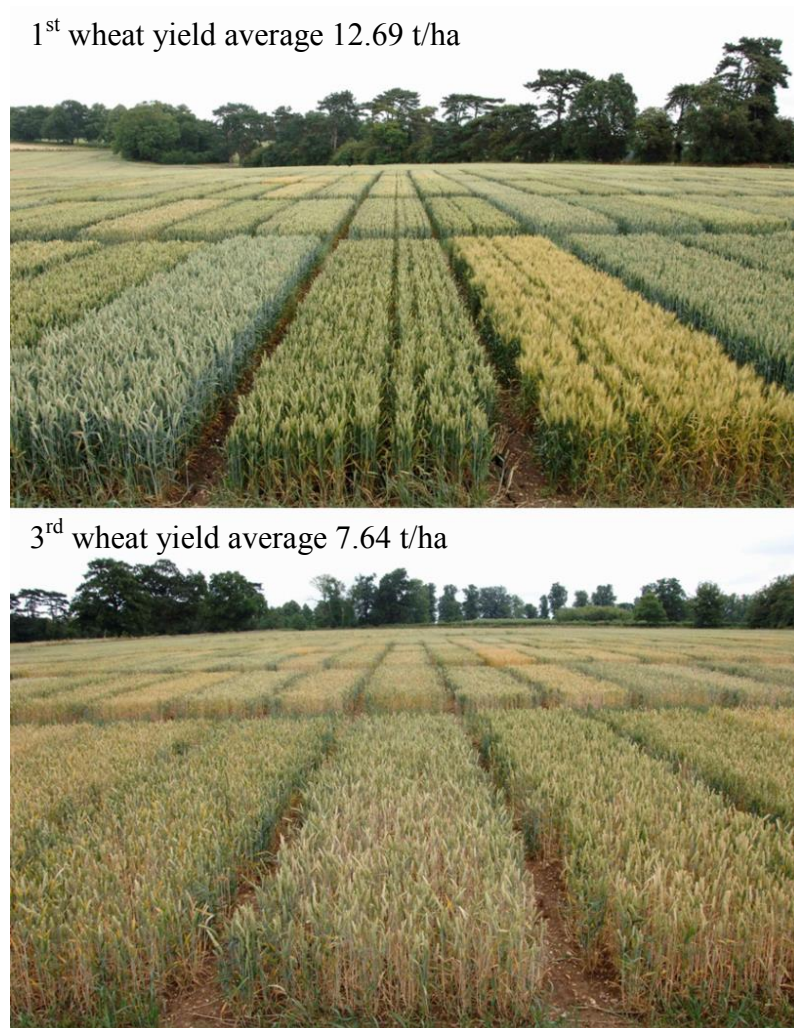


Figure 6.2. Photographs of the first wheat (09/R/WW/916; top picture) and third wheat (09/R/WW/917; bottom picture) variety field trials on the Rothamsted farm. Both trials sown on 09th October 2008. Photographs taken on 08th July 2009. Above ground symptoms of severe take-all disease (prematurely ripened and stunted plants) visible in the third wheat trial.

Only a relatively weak negative correlation was detected between mean variety yields and the take-all patch score in the third wheat field trial ($R_s = -0.37$, $P = 0.01$, $n = 45$;

Figure 6.3). There was no significant correlation between the take-all index and yield ($R_s = -0.24$, $P = 0.12$, $n = 45$; Figure 6.4).

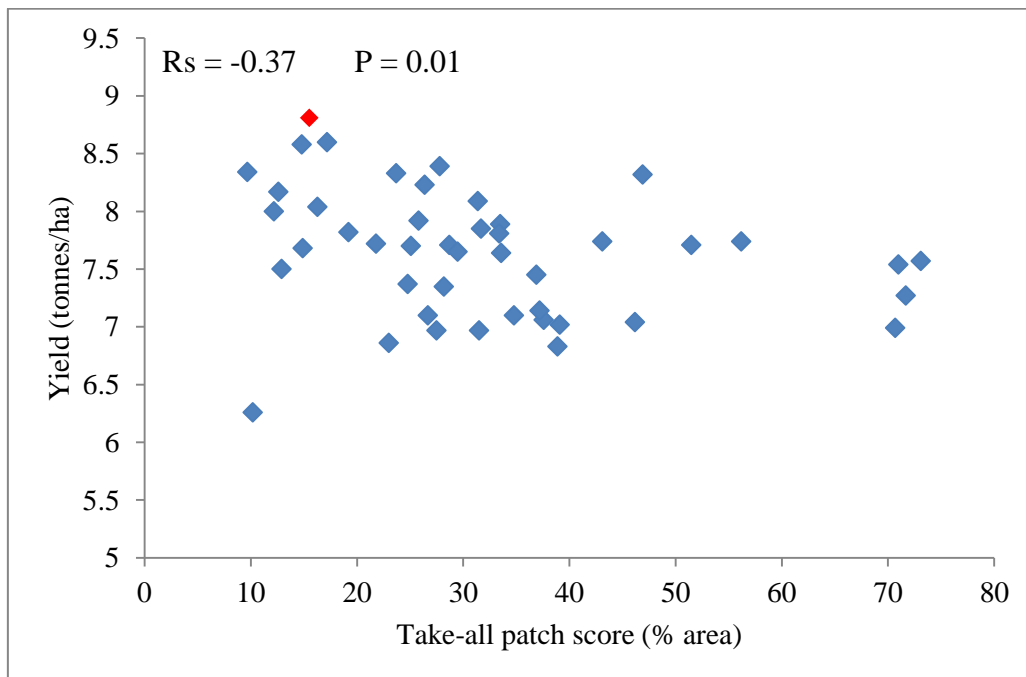


Figure 6.3. Correlation between the mean varietal take-all patch score and yields in the 2009 third wheat field trial (09/R/WW/917). Red diamond = winter wheat variety Hereford, with the highest 3rd wheat yield.

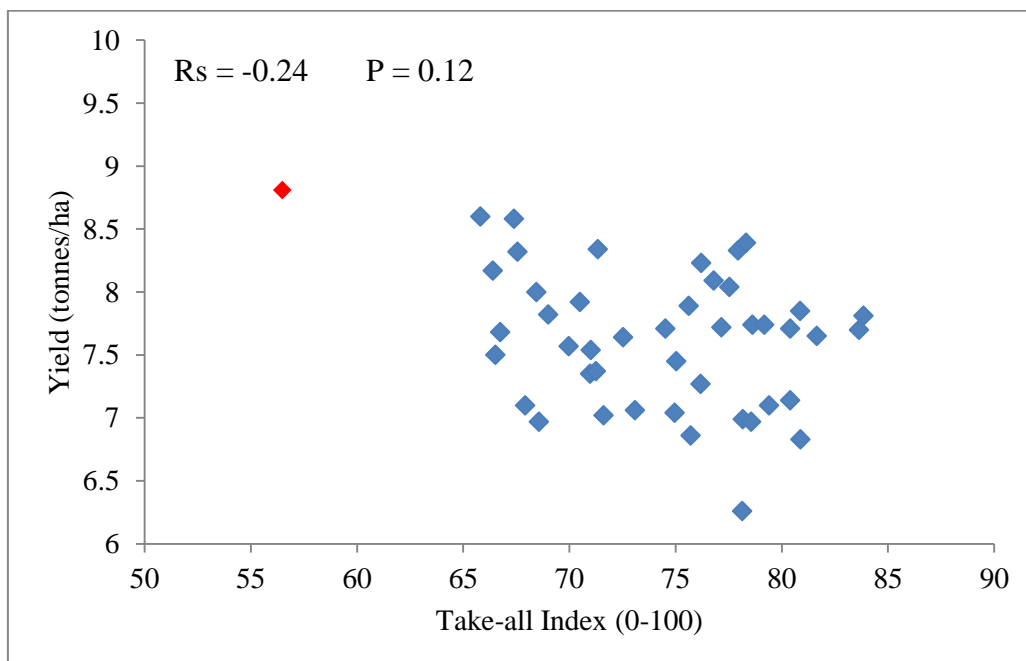


Figure 6.4. Correlation between the mean variety take-all index and yields in the 2009 third wheat field trial (09/R/WW/917). Red diamond = winter wheat variety Hereford, with the highest 3rd wheat yield and lowest take-all index out of the varieties tested.

The percentage yield loss, calculated to give a better representation of the yield effect of take-all on different varieties, was also not strongly correlated with the take-all patch score ($R_s = 0.26$, $P = 0.09$, $n = 45$; Figure 6.5) and take-all index ($R_s = 0.22$, $P = 0.14$, $n = 45$; Figure 6.6). Although the winter wheat variety Hereford had the lowest take-all index and highest yield of all of the varieties in the third wheat trial (Table 6.5), it was also one of the highest yielding varieties in the first wheat trial so its percentage yield loss is not the lowest. Overall there was a significant relationship between variety yields in the first and third wheat field trials ($R_s = 0.54$, $P < 0.001$, $n = 45$).

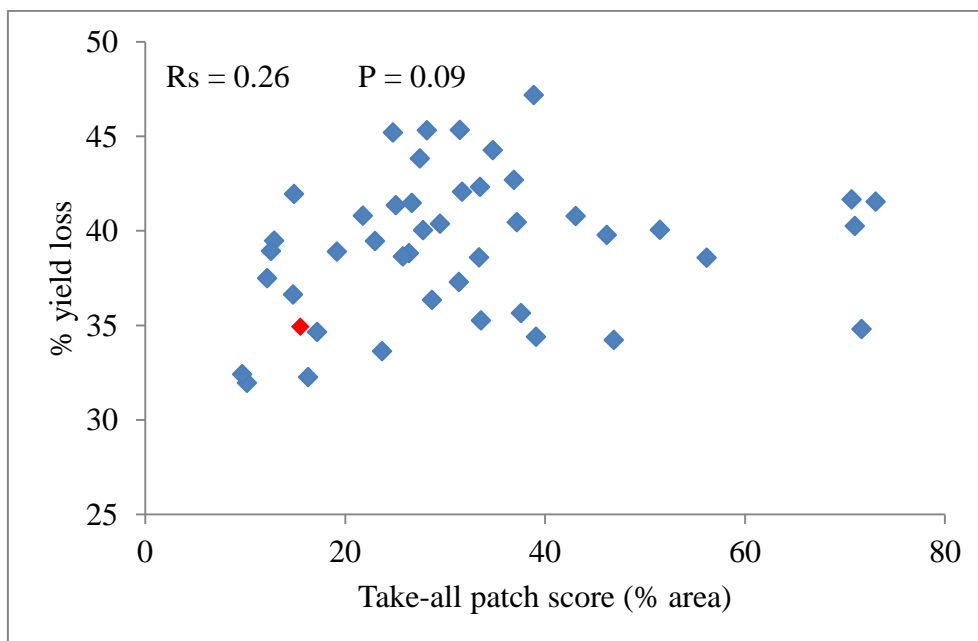


Figure 6.5. Correlation between the elite winter wheat variety take-all patch scores in the 2009 third wheat field trial (09/R/WW/917) and average percentage yield loss between the first (09/R/WW/916) and third wheat field trials. Red diamond = winter wheat variety Hereford, with the highest 3rd wheat yield, but percentage yield loss was similar to other varieties.

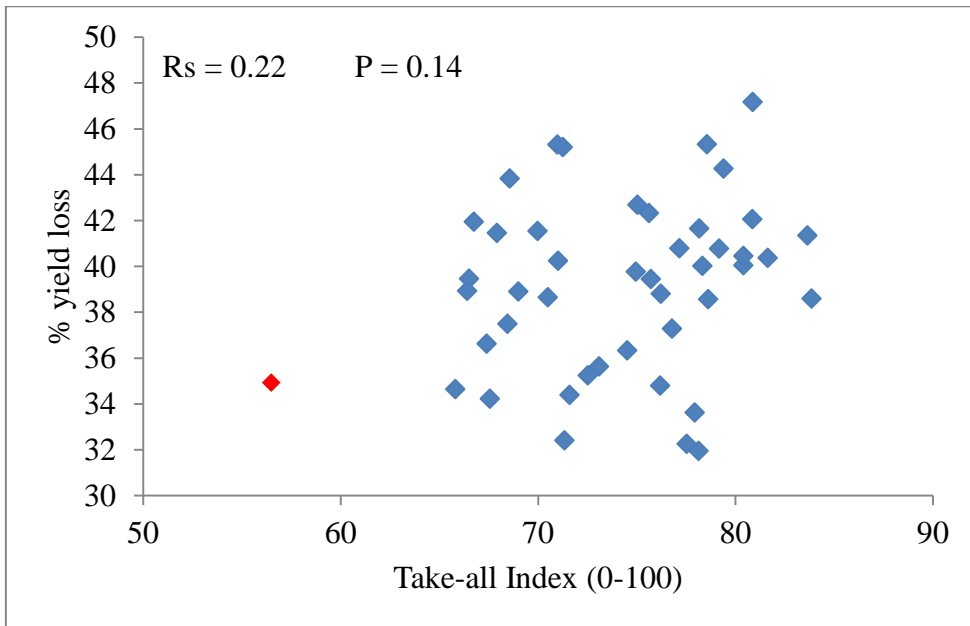


Figure 6.6. Correlation between the elite winter wheat variety take-all indexes in the 2009 third wheat field trial (09/R/WW/917) and average percentage yield loss between the first (09/R/WW/916) and third wheat field trials. Red diamond = winter wheat variety Hereford, with the lowest take-all index out of the varieties tested.

The 45 winter wheat varieties sown in the 3rd wheat field trial in 2009 (09/R/WW/917) were also assessed for their ability to build-up inoculum of the take-all fungus in the first wheat field trial in the same year (09/R/WW/916; Chapter 3). Significant varietal differences were detected in the amount of take-all inoculum built up by harvest ($P < 0.001$). A correlation analysis revealed no strong significant relationship between the ability of varieties to build-up inoculum of the take-all fungus during the first wheat crop and their susceptibility to take-all in the 3rd wheat field trial in 2009 ($R_s = 0.28$, $P = 0.06$, $n = 45$).

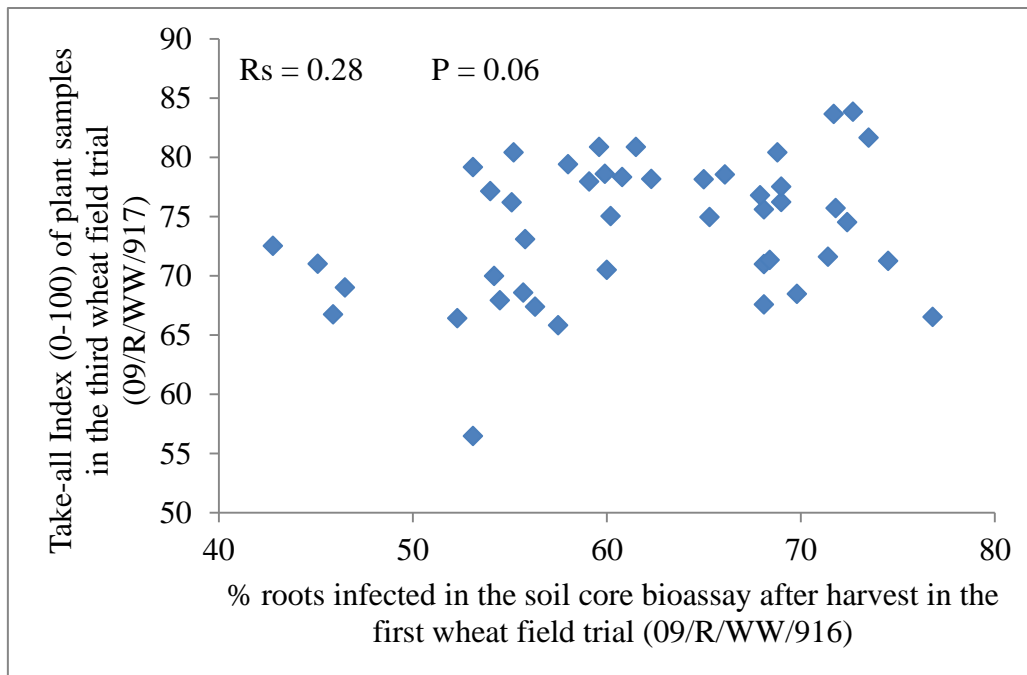


Figure 6.7. Relationship between the ability of elite wheat varieties to build-up take-all inoculum in the soil during a first wheat crop (09/R/WW/916) and their susceptibility to take-all infection in a third wheat high take-all disease pressure trial (09/R/WW/917).

An epidemiology study was carried out from April until July to chart take-all disease development for six selected varieties in the third wheat trial (09/R/WW/917). The study revealed a high take-all incidence in April 2009 with an average of 83.1% plants infected for all varieties (Tables 6.6a and 6.6b). There was a significant increase over the monthly sampling points to 99.3% plants infected at the final sampling point in July. There was no significant effect of variety on the percentage of plants infected with take-all or the number of seminal and crown roots infected with take-all. The number of seminal roots infected per plant roughly doubled for all varieties from April to July (Figure 6.8). While the number of crown roots infected per plant increased from 0.2 to 5.8. In June there was a slight trend towards a lower number of crown roots infected per

plant for the varieties Cordiale, Solstice and Xi19, although this was not significant (Figure 6.9). The six varieties were selected for the epidemiology study based on a limited number of previous pot and field tests, or based on their second wheat yield performance in the HGCA RL field trials. Solstice and Xi19 had previously shown low levels of take-all in a seedling pot test, while Einstein and Hereward had shown high levels of take-all in previous field and pot tests. However, in the epidemiology study there was no evidence that Solstice and Xi19 were less susceptible to take-all in the field than Einstein and Hereward. Cordiale and Robigus were chosen respectively as examples of good and poor second wheat varieties in terms of yield in HGCA RL trials. In the epidemiology study they were both equally susceptible to take-all, suggesting that the good reported second wheat yields of Cordiale are not related to lower susceptibility to take-all disease. Yields of Cordiale and Robigus in the third wheat trial were also not significantly different (Cordiale = 7.54 tonnes/ha, Robigus = 7.35 tonnes/ha; Table 6.5).

Table 6.6a. Take-all incidence from April to July for six varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).

Variety	Logit % plants with take-all (back-transformed mean)			
	April	May	June	July
Cordiale	1.58 (83.0)	1.87 (86.7)	4.51 (98.9)	4.76 (99.2)
Einstein	2.24 (90.4)	2.70 (93.7)	4.76 (99.2)	4.86 (99.3)
Hereward	1.66 (84.0)	3.80 (97.8)	3.88 (98.0)	4.67 (99.1)
Robigus	1.48 (81.4)	2.69 (93.7)	4.41 (98.8)	5.29 (99.5)
Solstice	1.48 (81.5)	1.93 (87.3)	3.70 (97.6)	4.93 (99.3)
Xi19	1.12 (75.4)	2.10 (89.1)	4.69 (99.1)	4.82 (99.2)
variety*month				
d.f.	52.98			
SED (logits)	0.913			
F Probability	0.865			

Table 6.6b. Take-all incidence from April to July for six varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).

Monthly mean	Logit % plants with take-all (back-transformed mean)	Variety mean	Logit % plants with take-all (back-transformed mean)
April	1.59 (83.1)	Cordiale	3.18 (96.0)
May	2.52 (92.5)	Einstein	3.65 (97.5)
June	4.32 (98.7)	Hereward	3.50 (97.1)
July	4.90 (99.3)	Robigus	3.47 (97.0)
		Solstice	3.01 (95.3)
		Xi19	3.18 (96.0)
d.f.	38.25	d.f.	15
SED (logits)	0.373	SED (logits)	0.456
F Probability	<.001	F Probability	0.724

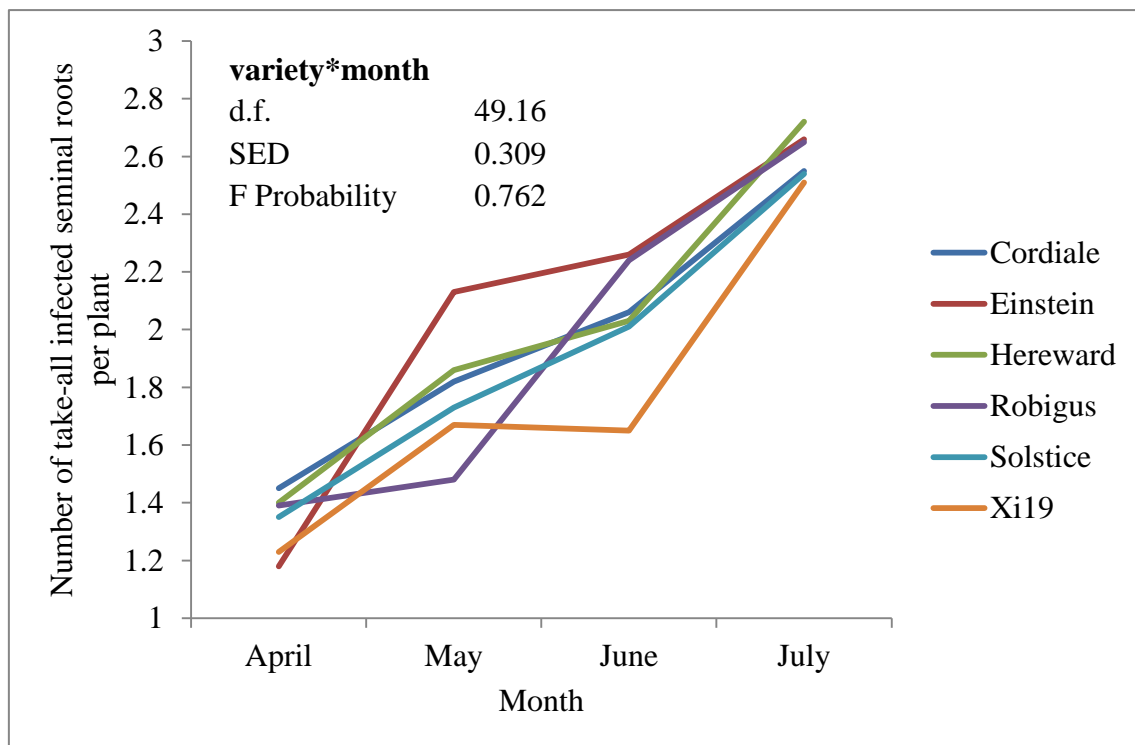


Figure 6.8. The number of take-all infected seminal roots per plant from April to July for six varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).

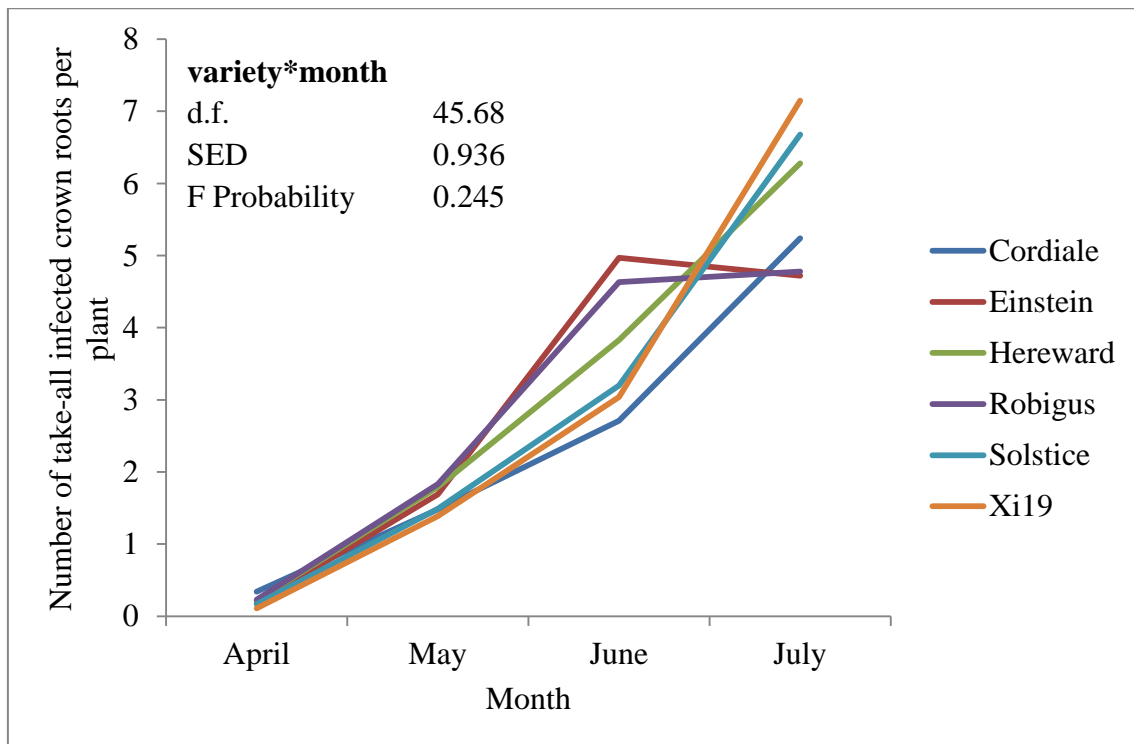


Figure 6.9. The number of take-all infected crown roots per plant from April to July for six varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).

The total number of seminal and crown roots were counted for a 10 plant sub-sample per plot at each monthly sampling point to assess root development for the six selected varieties. There was no significant varietal difference in the total number of seminal roots per plant (Table 6.7). Crown root development through the season increased from an average of 9.36 crown roots per plant in April to 17.45 in July (Table 6.8). There was a significant varietal effect on the average number of crown roots per plant, with the variety Solstice having the greatest average number of crown roots per plant across the sampling points ($P = 0.01$). There were no significant interactions between sampling date and variety ($P > 0.2$).

Table 6.7. Seminal root development from April to July for six winter wheat varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).

Monthly mean	Total number of seminal roots per plant	Variety mean	Total number of seminal roots per plant
April	4.23	Cordiale	3.74
May	3.56	Einstein	3.54
June	2.88	Hereward	3.48
July	3.53	Robigus	3.51
		Solstice	3.65
		Xi19	3.37
d.f.	39.95	d.f.	15
SED	0.133	SED	0.161
F Probability	<.001	F Probability	0.321

Table 6.8. Crown root development from April to July for six winter wheat varieties in the 2009 elite winter wheat and resistance to take-all field trial (09/R/WW/917).

Monthly mean	Total number of crown roots per plant	Variety mean	Total number of crown roots per plant
April	9.36	Cordiale	15.61
May	15.71	Einstein	13.76
June	18.28	Hereward	14.45
July	17.45	Robigus	15.95
		Solstice	16.75
		Xi19	14.69
d.f.	38.40	d.f.	15
SED	0.623	SED	0.724
F Probability	<.001	F Probability	0.010

The average number of seminal and crown roots per plant was also assessed for the same 6 varieties in the 1st wheat elite variety trial, to evaluate rooting ability in the absence of significant take-all infection (09/R/WW/916). These samples were taken in July. There was a similar number of seminal roots per plant in July for the 1st wheat trial (3.69 seminal roots per plant) compared with the third wheat trial (3.53 seminal roots per plant) (Table 6.9). However, there was on average 7.63 more crown roots per plant in the 1st wheat trial than the 3rd wheat trial. Varieties also differed significantly in the average number of crown roots per plant in July of the 1st wheat trial, although in this case it was the variety Robigus that had the greatest number of crown roots per plant.

Table 6.9. Mean number of seminal and crown roots per plant in July for six winter wheat varieties in the 2009 1st wheat field trial (09/R/WW/916).

Variety	Number of seminal roots per plant	Number of crown roots per plant
Cordiale	3.60	26.05
Einstein	3.63	22.98
Hereward	3.70	26.27
Robigus	3.70	28.20
Solstice	3.70	23.07
Xi19	3.80	23.88
d.f.	15	15
SED	0.212	1.568
F Probability	0.949	0.025
Grand Mean	3.69	25.08

2010

In 2010 the same 45 elite wheat varieties as 2009 were assessed in a third wheat field trial, except for three replacement varieties due to limited seed availability. Seed was unavailable due to a lack of recommendation after previous candidate years. Unfortunately there was no seed available of the winter wheat variety Hereford, which had the lowest take-all index in the 2009 trial. In contrast to 2009 significant varietal differences in the incidence (% plants infected) of take-all in the spring were detected in 2010 (range: 20% to 64% plants infected; $P = 0.003$) (Table 6.10). The wheat variety Hereward had the highest incidence of take-all (63.8% plants infected). There were also significant varietal differences in the severity of take-all ($P < 0.001$) with an average of 0.59 roots infected with take-all per plant. The incidence and severity of take-all were highly positively correlated (% plants infected and number of roots with take-all per plant: $R_s = 0.95$, $P = < 0.001$, $n = 45$). Only 0.04 crown roots were infected per plant on average and this was not significantly affected by variety ($P = 0.123$). In summer 2010 an average of 77.3% plants were infected with take-all and there was a mean take-all index of 21.56 (Table 6.10). No significant varietal differences were now detected. There were no significant correlations between disease in the spring and summer (Spearman's rank correlation analysis, data not shown). The spring and summer in 2010 were very dry and field trials across the Rothamsted Farm had clear drought symptoms with widespread leaf rolling, prematurely ripening ears and leaf senescence. Take-all patches in the 3rd wheat field trial (10/R/WW/1031) were not visible under these conditions so the trial was not scored for the extent of take-all patches.

Table 6.10. Take-all disease in the spring and summer of the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).

2010 Variety ¹	Spring				Summer		
	Logit % plants infected (BT means)	No. infected roots/plant	No. infected seminal roots/plant	No. infected crown roots/plant	TAI (0- 100)	Logit % plants infected (BT means)	Yield (tonnes/ha)
Alchemy	-0.68 (20.2)	0.31	0.28	0.03	22.13	0.43 (69.7)	7.86
Avalon	-0.08 (45.9)	0.77	0.73	0.03	22.08	0.84 (83.9)	8.06
Bantam	-0.42 (29.9)	0.39	0.38	0.02	22.01	0.64 (77.9)	9.33
Battalion	-0.19 (40.7)	0.57	0.56	0.01	22.57	0.58 (75.6)	8.44
Brompton	-0.36 (32.7)	0.50	0.45	0.04	26.58	0.79 (82.3)	8.89
Cadenza	0.10 (55.2)	0.99	0.96	0.03	20.68	0.80 (82.7)	8.14
Cassius	-0.67 (20.6)	0.41	0.37	0.04	21.68	0.58 (75.6)	9.38
Claire	-0.45 (28.5)	0.39	0.34	0.05	24.52	0.75 (81.2)	8.44
Conqueror	-0.34 (33.6)	0.53	0.47	0.05	32.48	1.09 (89.3)	9.32
Cordiale	-0.13 (43.5)	0.76	0.74	0.01	19.93	0.63 (77.4)	8.55
Duxford	-0.18 (41.0)	0.63	0.59	0.05	18.66	0.54 (74.0)	8.90
Edmunds	-0.54 (25.2)	0.46	0.39	0.07	21.88	0.66 (78.5)	8.82
Einstein	-0.04 (48.2)	0.80	0.70	0.09	21.62	0.65 (78.0)	8.57
Gallant	-0.14 (43.1)	0.68	0.63	0.05	20.92	0.72 (80.2)	8.53
Gladiator	-0.36 (32.5)	0.42	0.41	0.01	23.21	0.73 (80.8)	9.20
Grafton	0.05 (52.3)	0.77	0.70	0.07	20.77	0.64 (77.6)	9.20
Paragon ²	-0.58 (23.6)	0.37	0.36	0.01	20.76	0.75 (81.3)	7.52
Hereward	0.28 (63.8)	1.08	1.00	0.09	24.38	0.81 (83.1)	7.97
Humber	-0.26 (37.2)	0.65	0.59	0.05	27.61	0.70 (79.8)	9.51
Hyperion	-0.42 (29.8)	0.48	0.39	0.09	23.42	0.88 (84.7)	8.42
Invicta	-0.31 (35.0)	0.45	0.41	0.04	15.53	0.24 (61.2)	8.73
Istabraq	-0.52 (25.8)	0.30	0.28	0.02	17.30	0.36 (66.7)	9.07
JB Diego	-0.22 (39.3)	0.52	0.50	0.02	16.92	0.51 (72.9)	9.13
Ketchum	-0.16 (42.0)	0.61	0.58	0.03	22.42	0.67 (78.9)	9.38
Kipling	-0.25 (37.6)	0.60	0.58	0.02	20.03	0.49 (72.3)	8.96

Table 6.10. Continued

Variety ¹	Spring				Summer		
	Logit % plants infected (BT means)	No. infected roots/plant	No. infected seminal roots/plant	No. infected crown roots/plant	TAI (0-100)	Logit % plants infected (BT means)	Yield (tonnes/ha)
Lear	-0.42 (29.9)	0.37	0.34	0.03	15.84	0.31 (64.4)	8.86
Malacca	-0.08 (45.8)	0.62	0.58	0.03	19.05	0.68 (78.9)	8.24
Marksman	-0.06 (47.2)	0.65	0.62	0.03	26.05	0.79 (82.4)	8.42
Mascot	-0.27 (36.5)	0.46	0.46	0.01	22.60	0.74 (80.8)	8.17
Maris Widgeon ²	-0.15 (42.6)	0.62	0.58	0.04	20.86	0.63 (77.3)	7.30
Oakley	-0.43 (29.7)	0.36	0.34	0.02	23.41	0.64 (77.8)	8.82
Panorama	-0.44 (29.3)	0.40	0.38	0.02	20.77	0.53 (73.7)	8.55
Qplus	-0.34 (33.5)	0.52	0.46	0.06	23.26	0.48 (71.7)	7.97
Riband	-0.31 (34.7)	0.46	0.44	0.02	23.26	1.01 (87.8)	8.45
Robigus	-0.05 (47.5)	0.80	0.68	0.12	19.98	0.67 (78.8)	8.78
Scout	0.01 (50.4)	0.75	0.69	0.06	24.38	0.83 (83.5)	8.59
Sherborne	-0.03 (48.6)	0.89	0.79	0.11	22.10	0.73 (80.8)	8.90
Shogun	0.01 (50.5)	0.81	0.74	0.07	18.07	0.33 (65.6)	9.01
Soissons	-0.34 (33.5)	0.48	0.47	0.01	22.52	0.66 (78.4)	8.65
Solstice	0.13 (56.6)	1.02	0.91	0.12	20.02	0.49 (72.0)	8.30
Viscount	-0.24 (38.2)	0.51	0.52	-0.01	18.83	0.64 (77.8)	9.01
Glasgow ²	-0.37 (32.2)	0.45	0.43	0.02	24.93	0.55 (74.4)	9.18
Welford	-0.01 (49.3)	0.74	0.67	0.07	20.56	0.68 (79.1)	8.64
Xi19	0.06 (52.8)	0.88	0.77	0.11	17.62	0.62 (76.9)	8.22
Zebedee	-0.60 (23.0)	0.35	0.34	0.01	16.05	0.47 (71.3)	8.79
d.f.	44	44	44	44	44	44	44
SED Average	0.226	0.185	0.160	0.040	4.096	0.241	0.298
Wald statistic	89.10	102.67	112.61	57.91	55.15	43.68	257.21
F Probability	0.001	<0.001	<0.001	0.123	0.169	0.496	<0.001
Grand mean	-0.24 (38.6)	0.59	0.55	0.04	21.56	0.64 (77.3)	8.65

¹ **Bold** = varieties in epidemiology study. ² Replacement varieties.

Grain yield (tonnes/ha) was highly significantly different between varieties ($P < 0.001$) and an average yield of 8.65 tonnes/ha was recorded (Table 6.10). There was no significant relationship between take-all incidence or severity in the spring and yield (% plants infected in the spring and yield, $R_s = -0.19$, $P = 0.22$, $n = 45$; number of take-all infected roots per plant in the spring and yield, $R_s = -0.17$, $P = 0.25$, $n = 45$). There was also no significant correlation between the take-all index in the summer and yield (10/R/WW/1031) ($R_s = -0.03$, $P = 0.86$, $n = 45$; Figure 6.10).

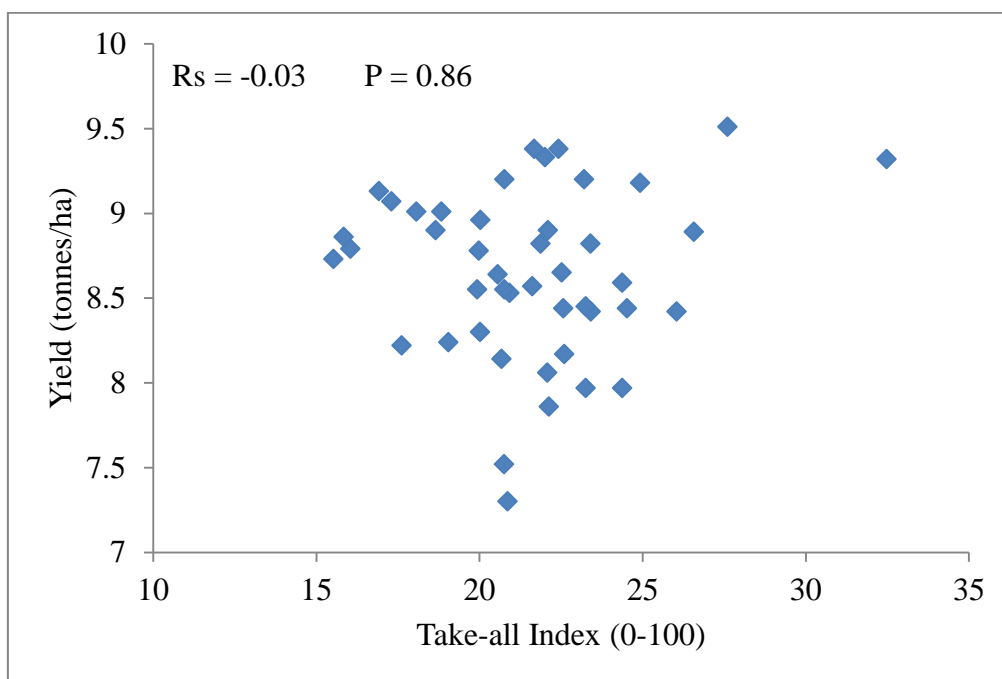


Figure 6.10. Correlation between the mean variety take-all index and yields in the 2010 third wheat field trial (10/R/WW/1031).

An average yield of 9.80 tonnes/ha was recorded for the same 45 varieties x 4 reps in the first wheat field trial (10/R/WW/1032). 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 ($R_s = 0.68$, $P < 0.001$, $n = 45$). There was no significant relationship between percentage yield loss and the take-all index ($R_s = -0.03$, $P = 0.82$, $n = 45$). Percentage yield loss was also not correlated with take-all incidence or severity in the spring (% plants infected in the spring and % yield loss, $R_s = -0.01$, $P = 0.92$, $n = 45$; number of take-all infected roots per plant in the spring and % yield loss, $R_s = -0.05$, $P = 0.73$, $n = 45$).

The 45 winter wheat varieties sown in the 3rd wheat field trial in 2010 (10/R/WW/1031) were also assessed for their ability to build-up inoculum of the take-all fungus in a first

wheat field trial in the same year (10/R/WW/1032) (Chapter 3: Evaluation of the take-all inoculum build-up trait). In the first wheat field trial (10/R/WW/1032) there was no appreciable take-all inoculum build-up under all varieties (Less than 5 % roots infected in the soil core bioassay across the trial). No significant relationship was found between inoculum build-up in this first wheat field trial and the take-all index in the third wheat field trial under these conditions ($R_s = 0.12$, $P = 0.44$, $n = 45$).

In the 2010 epidemiology study there were on average 52.4% plants infected in April (Tables 6.11a & 6.11b). This increased to 65.7% by July. There was no significant effect of variety on the percentage of plant infected with take-all ($P = 0.851$). Both the number of take-all infected seminal and crown roots per plant generally increased during the season, although there was less than 1.5 crown roots per plant infected with take-all throughout the study period (Figures 6.11 & 6.12). This is in contrast to 2009 when around 5-7 roots were infected per plant by July. The slow progression of take-all disease was presumably the result of the very dry weather in the spring and summer of 2010. This also restricted the build-up of take-all inoculum in the 1st wheat field trial in the same year (10/R/WW/1032; Chapter 3). As in the 2009 3rd wheat trial there was no significant effect of variety on the number of seminal or crown roots infected per plant ($P > 0.45$). In 2010 the varieties Avalon and Cadenza were added to the six original varieties selected for the epidemiology study. Avalon and Cadenza were selected based on their consistent contrasting abilities to build-up inoculum of the take-all fungus during a first wheat crop (Avalon = high TAB, Cadenza = low TAB; Chapter 3). In the epidemiology study there was no evidence that the take-all susceptibility of Avalon and Cadenza was different to each other or the other 6 varieties in the study. As in 2009 take-all disease and yields of the ‘good’ second wheat Cordiale and ‘poor’ second wheat Robigus were not significantly different when measured in the third wheat field trial (Table 6.10).

Table 6.11a. Take-all incidence from April to July for eight varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).

Variety	Logit % plants with take-all (back-transformed mean)			
	Month			
	April	May	June	July
Avalon	-0.42 (39.8)	0.35 (58.6)	0.65 (65.8)	0.63 (65.1)
Cadenza	0.39 (59.5)	0.33 (58.2)	0.66 (65.9)	0.60 (64.5)
Cordiale	-0.01 (49.7)	0.59 (64.3)	0.47 (61.4)	0.97 (72.6)
Einstein	0.09 (52.3)	0.79 (68.8)	0.42 (60.2)	0.76 (68.2)
Hereward	0.57 (63.9)	0.49 (62.0)	1.03 (73.8)	0.65 (65.7)
Robigus	-0.02 (49.6)	-0.66 (34.2)	0.71 (67.0)	0.47 (61.6)
Solstice	0.04 (51.0)	0.03 (50.7)	0.75 (68.0)	0.49 (62.0)
Xi19	0.13 (53.3)	0.29 (57.3)	0.39 (59.6)	0.62 (65.0)
variety*month				
d.f.	62.82			
SED (logits)	0.511			
F Probability	0.612			

Table 6.11b. Take-all incidence from April to July for eight varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).

Monthly mean	Logit % plants with take-all (back-transformed mean)	Variety mean	Logit % plants with take-all (back-transformed mean)
April	0.10 (52.4)	Avalon	0.30 (57.5)
May	0.28 (56.9)	Cadenza	0.49 (62.1)
June	0.64 (65.4)	Cordiale	0.50 (62.3)
July	0.64 (65.7)	Einstein	0.52 (62.6)
		Hereward	0.69 (66.5)
		Robigus	0.13 (53.2)
		Solstice	0.33 (58.1)
		Xi19	0.36 (58.9)
d.f.	55.72	d.f.	16
SED (logits)	0.150	SED (logits)	0.355
F Probability	0.002	F Probability	0.851

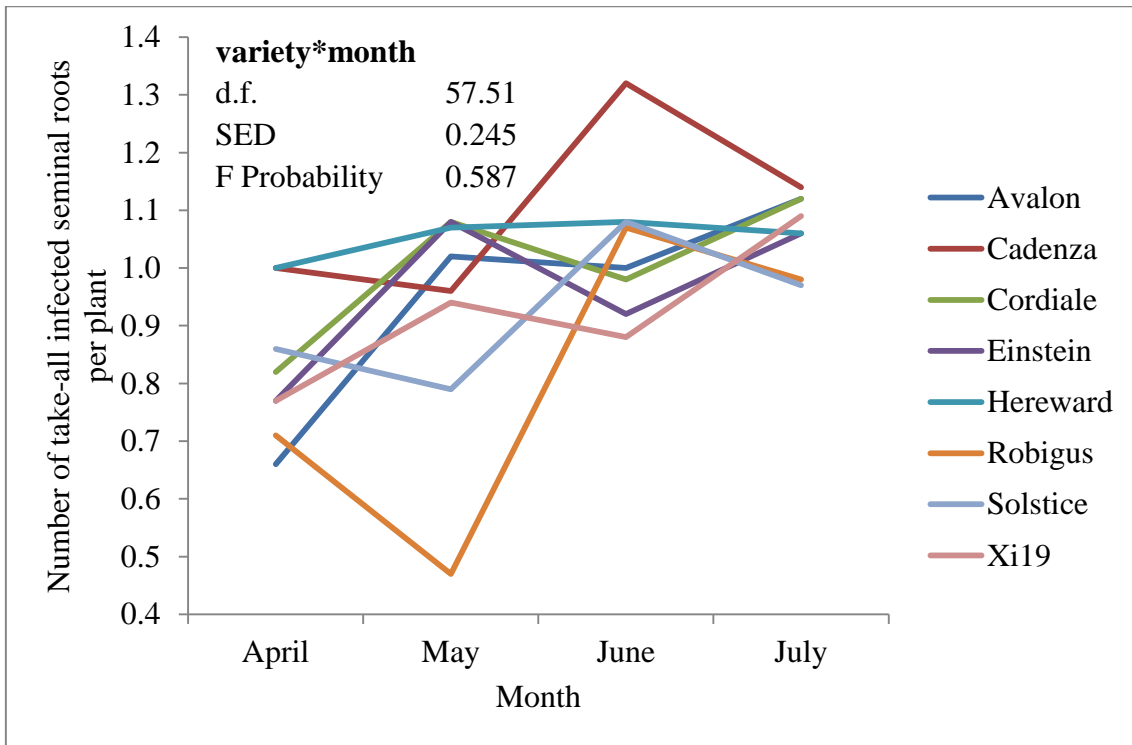


Figure 6.11. The number of take-all infected seminal roots per plant from April to July for eight varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).

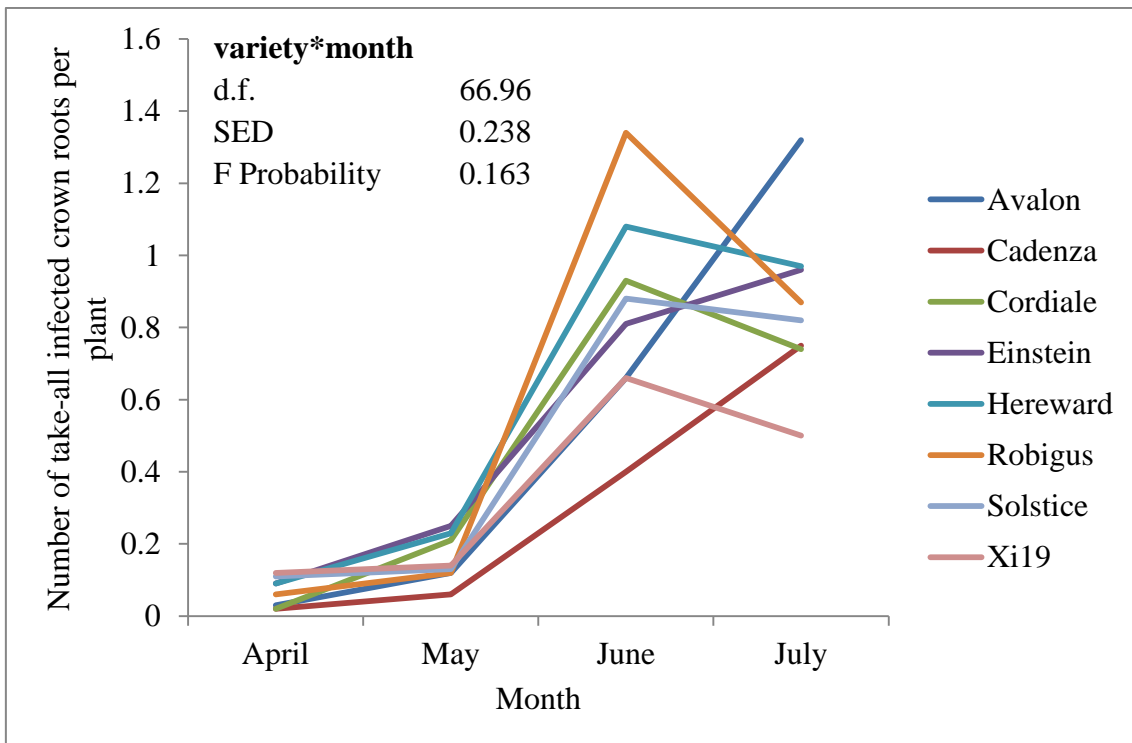


Figure 6.12. The number of take-all infected crown roots per plant from April to July for eight varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).

As in 2009 the total number of seminal and crown roots were counted for a 10 plant sub-sample per plot at each monthly sampling point to assess root development for the eight selected varieties. Seminal and crown root development in the 2010 3rd wheat field trial was similar to 2009. The number of seminal roots per plant was around 4 throughout the study period while the number of crown roots per plant increased from 8.39 to 18.55 from April to July (Tables 6.12 and 6.13). In contrast to 2009 there were significant varietal differences in the number of seminal roots per plant. Varietal differences were also detected in the number of crown roots per plant ($P < 0.001$; Table 6.13). However in 2010 the variety Robigus, instead of Solstice in 2009, had the greatest number of crown roots per plant. There were no significant interactions between sampling date and variety ($P > 0.2$).

Table 6.12. Seminal root development from April to July for eight winter wheat varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).

Monthly mean	Total number of seminal roots per plant	Variety mean	Total number of seminal roots per plant
April	4.41	Avalon	4.31
May	4.40	Cadenza	5.01
June	4.39	Cordiale	4.45
July	4.38	Einstein	4.10
		Hereward	4.13
		Robigus	4.39
		Solstice	4.47
		Xi19	4.32
d.f.	61.55	d.f.	21
SED	0.092	SED	0.153
F Probability	0.977	F Probability	<.001

Table 6.13. Crown root development from April to July for eight winter wheat varieties in the 2010 elite winter wheat and resistance to take-all field trial (10/R/WW/1031).

Monthly mean	Total number of crown roots per plant	Variety mean	Total number of crown roots per plant
April	8.39	Avalon	16.39
May	16.34	Cadenza	15.02
June	16.44	Cordiale	14.83
July	18.55	Einstein	14.05
		Hereward	14.55
		Robigus	16.77
		Solstice	14.77
		Xi19	13.06
d.f.	59.88	d.f.	21
SED	0.404	SED	0.565
F Probability	<.001	F Probability	<.001

The average number of seminal and crown roots per plant was also assessed in July for the same 8 varieties in the 1st wheat elite variety trial to evaluate root development in the absence of significant take-all infection (10/R/WW/1032) (Table 6.14). Seminal root numbers were similar in both the 1st and 3rd wheat trials. Unlike 2009 there was no big difference in the average number of crown roots per plant in the 1st and 3rd wheat trials (1st wheat trial mean = 19.07; 3rd wheat trial mean = 18.55).

Table 6.14. Mean number of seminal and crown roots per plant in July for eight winter wheat varieties in the 2010 1st wheat field trial (10/R/WW/1032).

Variety	Number of seminal roots per plant	Number of crown roots per plant
Avalon	4.30	20.07
Cadenza	4.95	19.55
Cordiale	4.63	18.75
Einstein	3.93	16.77
Hereward	4.43	17.80
Robigus	4.25	19.68
Solstice	4.38	21.62
Xi19	4.45	18.32
df	21	21
SED	0.309	1.397
F Probability	0.133	0.067
Grand Mean	4.41	19.07

2011

In 2011 the number of elite wheat varieties included in the 3rd wheat field trial was reduced to 10 based on the limited differences in incidence and severity of take-all between varieties in the two previous years and to limit the time spent assessing plant samples in autumn 2011, when funding for the PhD study would have stopped (PhD funded for three years from Oct 2008 to Oct 2011). 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. Two new Recommended List varieties were also included, Kingdom (Syngenta Seeds) and KWS Stirling (KWS UK Ltd).

In 2011 significant varietal differences in the incidence and severity of take-all disease were detected in the summer but not in the spring (Table 6.15). This is the same trend as in 2009. In the spring an average of 52.1% of plants were infected with take-all and 0.82 roots were infected on each plant. In the summer the variety Hereford had the lowest take-all index (24.7), with the average take-all index across all varieties of 41.6. There were no significant correlations between take-all disease in the spring and summer (Spearman's rank correlation analysis, data not shown). Another dry spring led to symptoms of leaf rolling and premature ripening so that take-all patches were not visible in the trial. An average grain yield (tonnes/ha) of 8.59 tonnes/ha was recorded (Table 6.15).

Table 6.15. Take-all disease in the spring and summer of the 2011 elite winter wheat and resistance to take-all field trial (11/R/WW/1114).

2011	Spring			Summer			
Variety ¹	Logit % plants infected (BT means)	No. infected roots/plant	No. infected seminal roots/plant	No. infected crown roots/plant	TAI (0-100)	Logit % plants infected (BT means)	Yield (tonnes/ha)
Duxford	0.03 (51.7)	0.80	0.74	0.06	38.4	1.02 (88.1)	9.15
Edmunds	0.19 (59.5)	0.87	0.76	0.11	46.0	1.56 (95.2)	8.36
Hereford	-0.04 (47.8)	0.67	0.57	0.10	24.7	0.50 (72.4)	9.04
Hereward	0.07 (53.5)	0.78	0.69	0.09	52.4	1.38 (93.6)	7.23
Invicta	0.14 (57.1)	1.01	0.87	0.14	56.5	1.40 (93.7)	8.53
Kingdom	0.13 (56.3)	0.87	0.83	0.04	32.6	0.82 (83.3)	8.56
Kipling	-0.22 (38.9)	0.68	0.60	0.08	44.3	1.05 (88.6)	8.85
KWS Stirling	0.17 (58.3)	1.02	0.98	0.04	40.9	1.16 (90.6)	8.40
Lear	-0.11 (44.7)	0.70	0.64	0.06	38.4	0.97 (87.0)	9.15
Solstice	0.07 (53.6)	0.81	0.74	0.07	41.5	1.04 (88.3)	8.59
d.f.	27	27	27	27	27	27	27
SED	0.248	0.232	0.212	0.044	7.760	0.280	0.521
F Probability	0.813	0.809	0.699	0.400	0.019	0.037	0.044
Grand Mean	0.04 (52.1)	0.82	0.74	0.08	41.6	1.09 (88.1)	8.59

¹ **Bold** = varieties in 2011 epidemiology study.

In contrast to 2009 and 2010, there was a significant correlation between yield and the percentage of plants infected with take-all in the summer ($R_s = -0.74$, $P = 0.01$, $n = 10$; Figure 6.13). Yield and the take-all index were also significantly negatively correlated ($R_s = -0.65$, $P = 0.04$, $n = 10$; Figure 6.14). This may be a consequence of the relatively low number of treatments (10 varieties) in the correlation analysis.

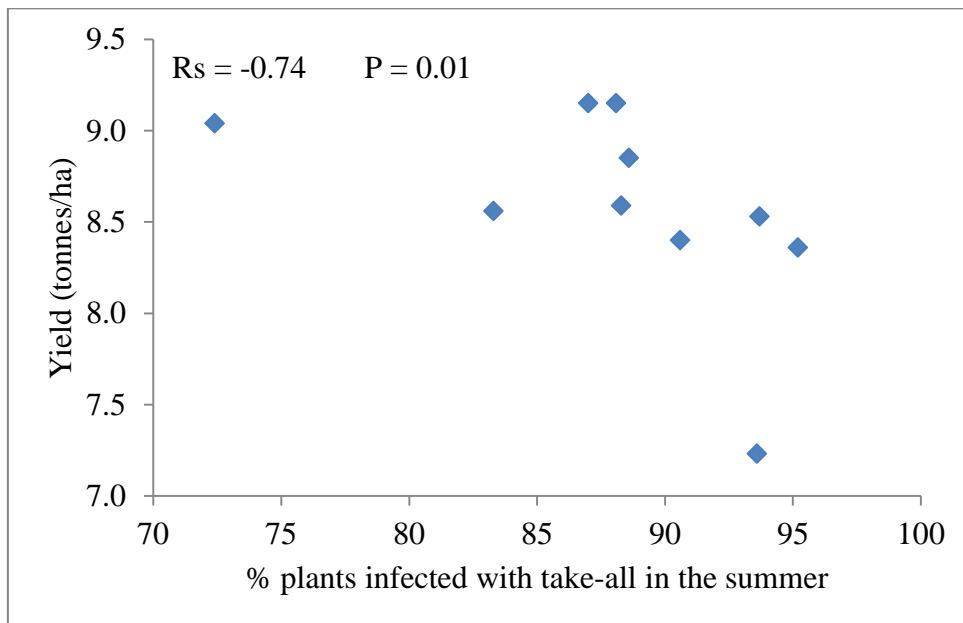


Figure 6.13. Correlation between the percentage of plants infected with take-all and variety yields in the 2011 third wheat field trial (11/R/WW/1114).

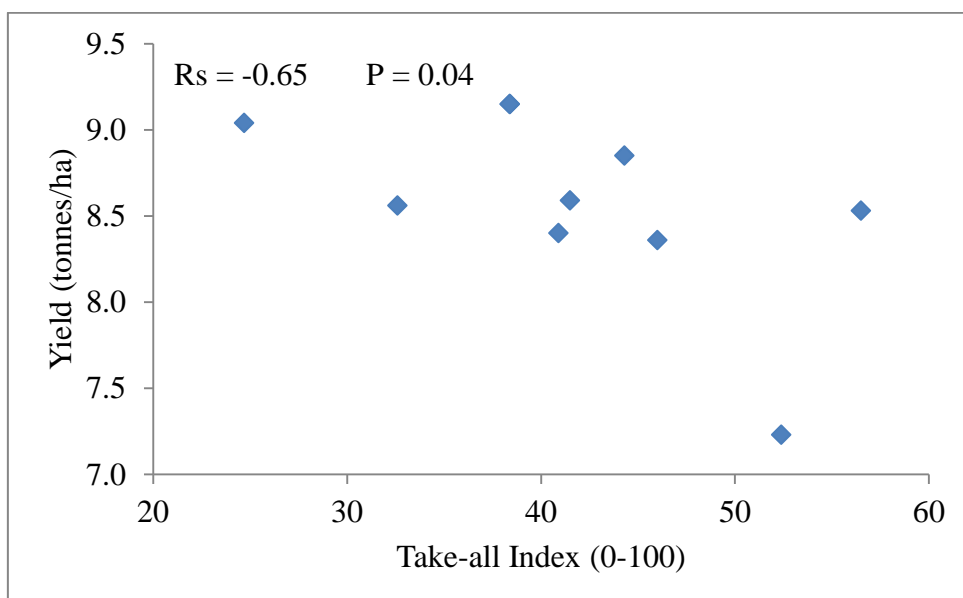


Figure 6.14. Correlation between the take-all index and variety yields in the 2011 third wheat field trial (11/R/WW/1114).

Eight of the ten varieties (excluding Hereford and KWS Stirling) were also included in the first wheat field trial evaluating take-all inoculum build-up of winter wheat varieties in 2011 (11/R/WW/1115). The average yield of these eight varieties in the first wheat trial was 11.86 tonnes/ha. The yield loss between the first wheat variety yields and the same eight varieties in the third wheat field trial was 27.9%. There was no significant correlation between percentage yield loss and the percentage of plants infected with take-all ($R_s = 0.52$, $P = 0.16$, $n = 8$) or the take-all index ($R_s = 0.56$, $P = 0.12$, $n = 8$) in the summer. In contrast to 2009 and 2010 no significant relationship was detected between variety first wheat yields and third wheat yields ($R_s = 0.56$, $P = 0.12$, $n = 8$).

Take-all inoculum build-up in 2011 first wheat field trial (11/R/WW/1115) was compromised by the carry-over of take-all inoculum through the break crop and the presence of *Phialophora* species (Chapter 3: Field evaluation of take-all inoculum build-up). Therefore it was 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.

In the 2011 epidemiology study the varieties Hereford and Hereward were chosen for evaluation based on their results in the 2009 field trial: Hereford has the lowest take-all index in the summer out of all 45 varieties and Hereward had one of the highest levels of take-all disease. In the 2011 epidemiology study there was a trend for a higher take-all incidence across the season for Hereward compared with Hereford, although there was no significant effect of variety in the repeated measures ANOVA (Table 6.16a and 6.16b). There was also no significant effect of variety on the average number of take-all infected seminal or crown roots per plant in the cross-season analysis (Take-all infected seminal roots, Hereford = 0.83, Hereward = 1.36, $P = 0.085$; Take-all infected crown roots, Hereford = 0.46, Hereward = 1.03, $P = 0.181$). However, there were close to significant interactions between month and variety for these two variables (Figures 6.15 and 6.16), with Hereford having less take-all infected seminal roots in June and July and less take-all infected crown roots in July than Hereward.

Table 6.16a. Take-all incidence from March to July for two varieties in the 2011 elite winter wheat and resistance to take-all field trial (11/R/WW/1114).

Variety	Logit % plants with take-all (back-transformed mean)				
	Month				
	March	April	May	June	July
Hereford	-1.05 (26.0)	-0.11 (47.3)	-0.16 (46.1)	1.40 (80.3)	0.83 (69.6)
Hereward	-0.45 (38.8)	0.12 (53.0)	0.31 (57.7)	2.90 (94.8)	3.60 (97.3)
variety*month					
d.f.	10.10				
SED (logits)	0.679				
F Probability	0.068				

Table 6.16b. Take-all incidence from March to July for two varieties in the 2011 elite winter wheat and resistance to take-all field trial (11/R/WW/1114).

Monthly mean	Logit % plants with take-all (back-transformed mean)	Variety mean	Logit % plants with take-all (back-transformed mean)
March	-0.75 (32.1)	Hereford	0.18 (54.6)
April	0.00 (50.1)	Hereward	1.29 (78.5)
May	0.08 (51.9)		
June	2.15 (89.6)		
July	2.21 (90.1)		
d.f.	10.85	d.f.	3
SED (logits)	0.392	SED (logits)	0.464
F Probability	<.001	F Probability	0.097

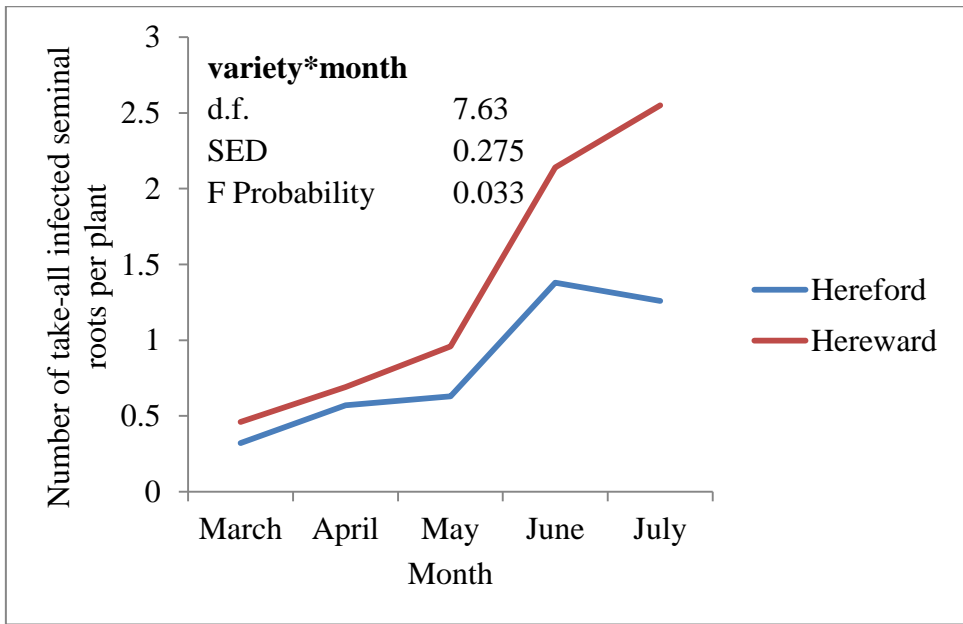


Figure 6.15. The number of take-all infected seminal roots per plant from March to July for two varieties in the 2011 elite winter wheat and resistance to take-all field trial (11/R/WW/1114).

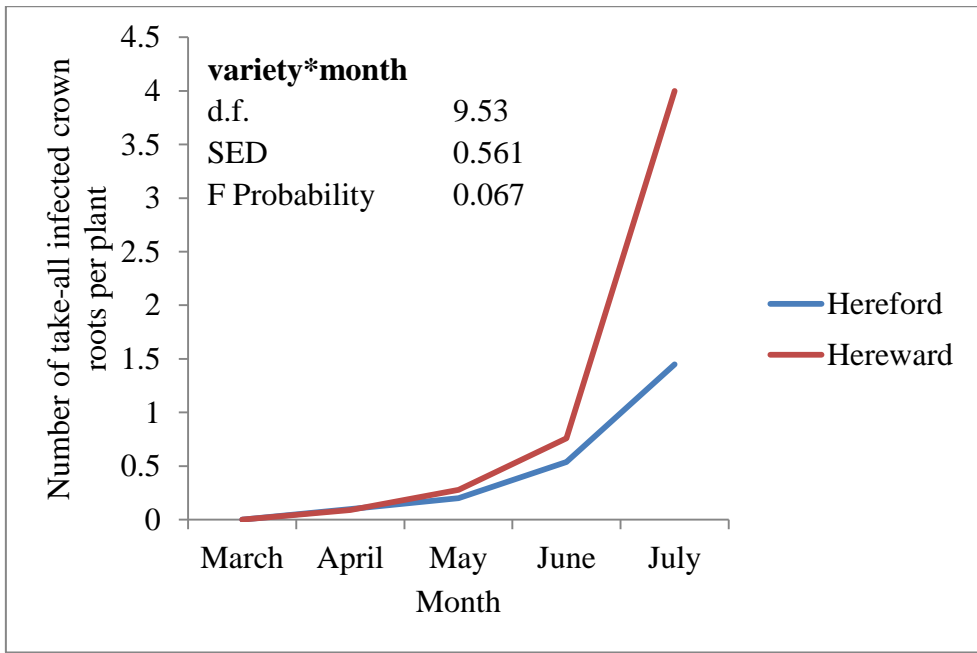


Figure 6.16. The number of take-all infected crown roots per plant from March to July for two varieties in the 2011 elite winter wheat and resistance to take-all field trial (11/R/WW/1114).

Seminal and crown root development was similar to 2009 and 2010 with around four seminal roots per plant throughout the spring and summer and an increase from 2 to 17 crown roots per plant from March until July for both Hereford and Hereward together (Tables 6.17 and 6.18). However, Hereford had a significantly higher number of crown

roots per plant throughout the season ($P = 0.01$). The variety Hereford was not included in the 2011 1st wheat field trial (11/R/WW/1115). However, seminal and crown root counts on the variety Hereward in the 1st wheat trial in July were similar to the root counts in the third wheat trial with an average 4.18 seminal roots and 15.75 crown roots per plant (compared with 4.21 seminal roots and 15.21 crown roots per plant in the third wheat trial in July).

Table 6.17. Seminal root development from March to July for the wheat varieties Hereford and Hereward in the 2011 elite winter wheat and resistance to take-all 3rd wheat field trial (11R/WW/1114).

Monthly mean	Total number of seminal roots per plant	Variety mean	Total number of seminal roots per plant
March	4.79	Hereford	4.51
April	4.04	Hereward	4.41
May	4.53		
June	4.69		
July	4.26		
d.f.	13.35	d.f.	3
SED	0.140	SED	0.080
F Probability	0.002	F Probability	0.330

Table 6.18. Crown root development from March to July for the wheat varieties Hereford and Hereward in the 2011 elite winter wheat and resistance to take-all 3rd wheat field trial (11R/WW/1114).

Monthly mean	Total number of crown roots per plant	Variety mean	Total number of crown roots per plant
March	2.53	Hereford	12.96
April	6.58	Hereward	9.75
May	13.98		
June	16.53		
July	17.17		
d.f.	10.51	d.f.	3
SED	0.380	SED	0.552
F Probability	<.001	F Probability	0.010

6.3.2. Combined year comparison

A combined year analysis of the spring and summer disease variables was carried out using a REML analysis. The percentage plants infected with take-all in the spring and summer were transformed using logits before analysis, to ensure equal variance. In the REML analysis the number of infected roots, seminal roots and crown roots per plant in the spring were also transformed to stabilise the variance. Transformation was indicated by examining the residual diagnostic plots and the square root transformation was used. 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 tables of 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 sampling points, suggesting that modern hexaploid wheat varieties differ in their susceptibility to take-all disease (Table 6.19). However in the individual year analyses above the susceptibility of varieties to take-all was not generally related to percentage yield loss. In the combined year analysis only the number of infected crown roots per plant in the spring was not significantly different between varieties ($P = 0.287$). In the spring analysis the percentage of plants with take-all ranged from 43% to 73%. Four varieties had less than 50% plants infected: Cassius, Paragon, Hyperion and Alchemy. There was on average 0.93 roots infected per plant in the spring. In the combined analysis of summer samples all of the varieties, except Hereford and Kingdom, had over 90% plants infected with take-all. These two varieties also had the lowest take-all index calculated from the proportion of take-all disease on the summer plant samples (Table 6.19). Hereford was included in two trial years (2009 and 2010) and Kingdom was only in the final trial year (2011).

Table 6.19. Combined year analysis of take-all disease of fifty winter wheat varieties in the spring and summer of three third wheat field trials (09/R/WW/917, 10/R/WW/1031 and 11/R/WW/1114).

Variety	Spring			Summer		
	Logit % plants with take-all (BT ¹ means)	Sqrt ² number of infected roots per plant (BT means)	Sqrt ² number of infected seminal roots per plant (BT means)	Sqrt ² number of infected crown roots per plant (BT means)	TAI (0-100)	Logit % plants with take-all (BT means)
Alchemy	-0.01 (49.6)	0.92 (0.85)	0.88 (0.77)	0.28 (0.08)	45.19	1.36 (93.9)
Avalon	0.27 (63.3)	0.99 (0.97)	0.94 (0.88)	0.24 (0.06)	46.61	1.58 (96.0)
Bantam	0.23 (61.5)	0.96 (0.93)	0.88 (0.77)	0.33 (0.11)	45.78	1.25 (92.4)
Battalion	0.27 (63.1)	0.98 (0.96)	0.92 (0.85)	0.28 (0.08)	50.09	1.49 (95.1)
Brompton	0.21 (60.5)	0.96 (0.93)	0.90 (0.82)	0.31 (0.10)	54.26	1.53 (95.6)
Cadenza	0.40 (68.8)	1.14 (1.30)	1.08 (1.16)	0.28 (0.08)	46.71	1.54 (95.6)
Cassius	-0.12 (43.8)	0.85 (0.72)	0.78 (0.62)	0.30 (0.09)	44.18	1.30 (93.1)
Claire	0.03 (51.4)	0.89 (0.79)	0.83 (0.69)	0.32 (0.10)	51.43	1.58 (95.9)
Conqueror	0.10 (55.2)	0.92 (0.84)	0.85 (0.72)	0.31 (0.10)	54.37	1.50 (95.3)
Cordiale	0.26 (62.5)	1.04 (1.08)	0.97 (0.95)	0.29 (0.09)	45.01	1.48 (95.1)
Duxford	0.24 (61.9)	1.00 (0.99)	0.94 (0.89)	0.29 (0.08)	42.35	1.23 (92.2)
Edmunds	0.19 (59.6)	1.03 (1.06)	0.94 (0.89)	0.38 (0.14)	50.14	1.58 (95.9)
Einstein	0.45 (71.3)	0.97 (0.94)	0.90 (0.81)	0.33 (0.11)	48.03	1.53 (95.5)
Gallant	0.34 (66.5)	0.98 (0.96)	0.94 (0.89)	0.26 (0.07)	47.86	1.48 (95.0)
Gladiator	0.07 (53.5)	0.83 (0.68)	0.80 (0.64)	0.19 (0.03)	51.07	1.50 (95.3)
Glasgow	0.09 (54.5)	0.87 (0.76)	0.83 (0.69)	0.23 (0.05)	50.88	1.33 (93.4)
Grafton	0.39 (68.6)	0.97 (0.95)	0.92 (0.84)	0.26 (0.07)	45.01	1.37 (94.0)
Hereford	0.16 (58.0)	0.88 (0.78)	0.81 (0.66)	0.32 (0.10)	28.64	0.70 (80.2)
Hereward	0.44 (70.6)	1.05 (1.11)	0.99 (0.98)	0.35 (0.12)	50.12	1.48 (95.0)
Humber	0.40 (68.9)	1.05 (1.11)	1.01 (1.03)	0.28 (0.08)	51.28	1.42 (94.5)
Hyperion	-0.01 (49.3)	0.84 (0.71)	0.79 (0.62)	0.28 (0.08)	49.59	1.36 (93.8)
Invicta	0.20 (60.0)	1.02 (1.04)	0.93 (0.86)	0.39 (0.16)	43.10	1.39 (94.1)
Istabraq	0.03 (51.7)	0.85 (0.73)	0.83 (0.68)	0.21 (0.04)	42.71	1.18 (91.4)

Table 6.19. Continued

Variety	Spring			Summer		
	Logit % plants with take-all (BT ¹ means)	Sqrt ² number of infected roots per plant (BT means)	Sqrt ² number of infected seminal roots per plant (BT means)	Sqrt ² number of infected crown roots per plant (BT means)	TAI (0-100)	Logit % plants with take-all (BT means)
JB Diego	0.27 (63.1)	0.97 (0.95)	0.92 (0.84)	0.28 (0.08)	45.88	1.47 (94.9)
Ketchum	0.19 (59.2)	0.97 (0.94)	0.91 (0.83)	0.30 (0.09)	49.57	1.44 (94.7)
Kingdom	0.32 (65.6)	1.03 (1.05)	0.99 (0.98)	0.22 (0.05)	33.39	0.96 (87.3)
Kipling	0.12 (56.2)	0.94 (0.88)	0.90 (0.80)	0.25 (0.06)	49.15	1.26 (92.5)
KWS Stirling	0.36 (67.5)	1.09 (1.18)	1.06 (1.11)	0.22 (0.05)	41.62	1.30 (93.1)
Lear	0.03 (51.7)	0.89 (0.79)	0.84 (0.71)	0.27 (0.08)	40.85	1.30 (93.0)
Malacca	0.34 (66.4)	1.01 (1.02)	0.95 (0.90)	0.28 (0.08)	45.42	1.50 (95.2)
Maris Widgeon	0.27 (63.1)	0.99 (0.98)	0.93 (0.86)	0.35 (0.12)	47.02	1.42 (94.5)
Marksman	0.29 (64.2)	0.96 (0.93)	0.89 (0.80)	0.29 (0.08)	51.84	1.56 (95.7)
Mascot	0.43 (70.3)	0.92 (0.85)	0.90 (0.81)	0.17 (0.03)	50.84	1.45 (94.8)
Monty	0.04 (51.9)	0.89 (0.80)	0.84 (0.71)	0.27 (0.07)	57.32	1.53 (95.6)
Oakley	0.10 (55.2)	0.89 (0.79)	0.83 (0.69)	0.30 (0.09)	50.36	1.54 (95.6)
Panorama	0.10 (55.2)	0.90 (0.82)	0.87 (0.76)	0.24 (0.06)	48.22	1.19 (91.6)
Paragon	-0.14 (43.3)	0.80 (0.64)	0.76 (0.57)	0.18 (0.03)	46.31	1.53 (95.5)
Qplus	-0.07 (46.6)	0.86 (0.75)	0.79 (0.62)	0.30 (0.09)	48.27	1.34 (93.6)
Riband	0.20 (59.7)	0.87 (0.76)	0.79 (0.62)	0.31 (0.10)	50.19	1.63 (96.3)
Robigus	0.30 (64.8)	1.03 (1.05)	0.95 (0.90)	0.34 (0.12)	45.15	1.42 (94.5)
Scout	0.48 (72.5)	1.14 (1.30)	1.07 (1.15)	0.36 (0.13)	50.71	1.62 (96.2)
Sherborne	0.28 (63.6)	1.08 (1.17)	0.98 (0.96)	0.41 (0.17)	46.25	1.45 (94.8)
Shogun	0.41 (69.6)	1.04 (1.08)	0.96 (0.92)	0.36 (0.13)	42.37	1.19 (91.5)
Soissons	0.10 (54.9)	0.89 (0.78)	0.84 (0.70)	0.25 (0.06)	49.54	1.44 (94.7)
Solstice	0.34 (66.6)	1.04 (1.08)	0.98 (0.96)	0.32 (0.10)	43.89	1.12 (90.4)
Viscount	0.10 (55.2)	0.88 (0.78)	0.85 (0.73)	0.17 (0.03)	44.89	1.44 (94.7)
Walpole	0.13 (56.7)	1.09 (1.19)	1.00 (0.99)	0.39 (0.15)	44.37	1.38 (94.0)
Welford	0.43 (70.3)	1.02 (1.03)	0.93 (0.86)	0.37 (0.13)	50.06	1.58 (95.9)

Table 6.19. Continued

Variety	Spring			Summer		
	Logit % plants with take-all (BT ¹ means)	Sqrt ² number of infected roots per plant (BT means)	Sqrt ² number of infected seminal roots per plant (BT means)	Sqrt ² number of infected crown roots per plant (BT means)	TAI (0-100)	Logit % plants with take-all (BT means)
Xi19	0.27 (63.3)	0.98 (0.96)	0.92 (0.84)	0.29 (0.09)	42.80	1.43 (94.6)
Zebedee	0.03 (51.7)	0.91 (0.83)	0.88 (0.78)	0.24 (0.06)	46.55	1.38 (94.0)
d.f.	49	49	49	49	49	49
SED (Average)	0.193	0.098	0.086	0.080	3.538	0.156
Wald statistic	71.96	69.38	77.14	54.98	149.02	115.63
F Probability	0.032	0.046	0.013	0.287	<0.001	<0.001
Grand mean	0.21 (60.0)	0.96 (0.93)	0.90 (0.82)	0.29 (0.09)	46.94	1.40 (93.9)

¹BT = back-transformed.

²Sqrt = square root transformation.

6.3.3. Response of winter wheat varieties to take-all in seedling pot tests

Before use in pot tests *Ggt* isolates were classified molecularly into two sub populations (A/B) using a specific PCR assay (Freeman et al., 2005), and were also classified in fungicide sensitivity tests as resistant or sensitive to the fungicide silthiofam (Chapter 5: Characterisation of a new *Ggt* isolate collection).

In 2009 an average of 46.9% and 59.9% roots were infected on wheat seedlings with the silthiofam resistant (R) and silthiofam sensitive (S) isolate mixtures respectively (The R and S isolates used were all A type based on molecular classification) (Tables 6.20 and 6.21). Seeds of the wheat variety Riband did not germinate in the pot tests in 2009 and so this variety was excluded from the analysis. New Riband seed was obtained for the 2011 pot test with the B type (molecular sub population) *Ggt* isolate mixture. The four B type isolates used were all fully sensitive to the fungicide silthiofam on treated wheat seed. Despite using a strong dilution of *Ggt* sand/maizemeal to silver sand (1:50) and mixing the pot test soil with sand there was only an average of 7.7% roots infected on the seedlings in the B type isolate pot test (Table 6.22). This could be because the B type isolates are inherently less pathogenic or because they do not grow well in artificial culture of sand/maizemeal. The Hereward seedlings in control pots without addition of artificial *Ggt* inoculum were free from take-all in all of the pot tests.

The total number of roots per plant varied significantly by variety in all three pot tests (Tables 6.20, 6.21 and 6.22). However this was not correlated with the percentage of roots infected (S isolate pot test: $R_s = -0.03$, $P = 0.85$, $n = 44$; R isolate pot test: $R_s = 0.02$, $P = 0.92$, $n = 44$; B isolate pot test: $R_s = 0.13$, $P = 0.40$, $n = 45$). There were no significant differences in susceptibility to take-all between varieties using the silthiofam resistant isolate mixture ($P = 0.797$). However, significant varietal differences were detected in the silthiofam sensitive and B type isolate mixture pot tests ($P < 0.001$ in both tests). There was no significant relationship between varietal performance in the silthiofam sensitive and B type isolate pot test ($R_s = 0.10$, $P = 0.53$, $n = 44$; Figure 6.17). Two varieties Solstice and Xi19 were previously identified by Richard Gutteridge as displaying some resistance to take-all in a limited number of seedling pot tests. In the three PhD pot tests below neither Solstice nor Xi19 stand out as better than the majority of other hexaploid wheats.

Interestingly in the lower disease pressure B type isolate pot test there was a greater range of susceptibilities to take-all in the tested varieties. Most varieties in this test

generally had quite a low percentage of roots infected with take-all (less than 10%). However, five varieties (Conqueror, Edmunds, Einstein, Invicta and Monty) had much higher levels of root infection (between 18 and 30% roots infected with take-all).

No significant relationships were found between the percentage roots infected at the seedling stage in the three pot tests and the take-all index of adult plants in the 2009 and 2010 field trials (Table 6.23). However, there were significant relationships detected between the results in the silthifam resistant and silthiofam sensitive pot tests and the take-all index of the eight varieties in common to the 2011 field trial (Table 6.23). At the seedling stage differences between these eight varieties were very much smaller, but in general the ranking of varieties was similar to the field.

Table 6.20. Susceptibility of elite winter wheat varieties to take-all infection in a seedling pot test using silthiofam resistant (R) *Ggt* isolates.

Variety	Logit % roots infected with take-all (back-transformed means)	Mean no. roots/plant	Mean no. take-all infected roots/plant
Alchemy	-0.09 (47.8)	7.04	3.43
Avalon	-0.23 (44.3)	6.74	3.06
Bantam	-0.22 (44.5)	7.08	3.27
Battalion	-0.35 (41.2)	6.06	2.62
Brompton	-0.28 (43.1)	6.66	2.91
Cadenza	-0.06 (48.4)	7.20	3.58
Cassius	-0.02 (49.6)	6.58	3.24
Claire	-0.11 (47.2)	6.62	3.13
Conqueror	-0.07 (48.3)	6.39	3.11
Cordiale	-0.36 (41.2)	6.74	2.82
Duxford	-0.22 (44.4)	6.10	2.81
Edmunds	-0.05 (48.8)	6.71	3.31
Einstein	-0.11 (47.2)	6.35	3.03
Gallant	-0.48 (38.1)	6.95	2.74
Gladiator	-0.12 (47.0)	6.01	2.85
Grafton	-0.17 (45.7)	6.31	2.94
Hereford	-0.29 (42.8)	6.38	2.74
Hereward	-0.14 (46.6)	5.39	2.54
Humber	0.19 (54.8)	6.68	3.63
Hyperion	0.15 (53.7)	5.94	3.23
Invicta	0.03 (50.7)	6.33	3.25
Istabraq	-0.06 (48.5)	6.87	3.38
JB Diego	-0.01 (49.8)	6.93	3.41
Ketchum	-0.22 (44.6)	6.63	2.99
Kipling	0.29 (57.1)	6.01	3.42
Lear	-0.53 (37.1)	6.42	2.48
Malacca	-0.26 (43.4)	7.02	3.12

Table 6.20. Continued

Variety	Logit % roots infected with take-all (back-transformed means)	Mean no. roots/plant	Mean no. take-all infected roots/plant
Marksman	-0.22 (44.4)	6.81	3.05
Mascot	-0.28 (42.9)	6.35	2.81
Monty	-0.10 (47.6)	6.77	3.26
Oakley	0.27 (56.8)	6.88	3.88
Panorama	0.17 (54.2)	7.35	4.00
Qplus	-0.30 (42.6)	6.66	2.84
Robigus	-0.22 (44.5)	6.21	2.78
Scout	0.07 (51.9)	7.07	3.66
Sherborne	0.39 (59.7)	6.66	3.98
Shogun	0.08 (52.1)	6.68	3.48
Soissons	-0.50 (37.7)	6.87	2.70
Solstice	-0.07 (48.3)	7.53	3.69
Viscount	-0.47 (38.6)	7.68	3.04
Walpole	-0.08 (48.1)	6.48	3.16
Welford	-0.17 (45.9)	7.20	3.32
Xi19	-0.21 (44.8)	6.93	3.18
Zebedee	-0.10 (47.6)	6.72	3.20
d.f.	176	176	176
SED	0.328	0.421	0.617
F Probability	0.797	<.001	0.899
Grand mean	-0.13 (46.9)	6.66	3.16

Table 6.21. Susceptibility of elite winter wheat varieties to take-all infection in a seedling pot test using silthiofam sensitive (S) *Ggt* isolates.

Variety	Logit % roots infected with take-all (back-transformed means)	Mean no. roots/plant	Mean no. take-all infected roots/plant
Alchemy	0.41 (60.2)	7.18	4.27
Avalon	0.30 (57.5)	7.42	4.27
Bantam	0.24 (55.9)	7.42	4.15
Battalion	-0.11 (47.3)	7.12	3.36
Brompton	0.15 (53.7)	7.20	3.85
Cadenza	0.15 (53.8)	7.27	3.91
Cassius	0.41 (60.1)	6.62	3.98
Claire	0.16 (53.9)	6.84	3.67
Conqueror	0.44 (60.9)	6.77	4.09
Cordiale	0.53 (62.8)	7.26	4.58
Duxford	0.26 (56.4)	6.47	3.65
Edmunds	0.71 (66.9)	7.42	4.94
Einstein	0.17 (54.2)	6.99	3.80
Gallant	0.00 (50.1)	7.79	3.93
Gladiator	0.30 (57.6)	6.94	4.00

Table 6.21. Continued

Variety	Logit % roots infected with take-all (back-transformed means)	Mean no. roots/plant	Mean no. take-all infected roots/plant
Grafton	0.38 (59.3)	6.73	3.99
Hereford	-0.07 (48.3)	6.20	2.96
Hereward	0.39 (59.7)	5.83	3.48
Humber	0.33 (58.2)	6.62	3.85
Hyperion	0.27 (56.6)	6.56	3.66
Invicta	0.65 (65.6)	6.89	4.41
Istabraq	0.61 (64.8)	6.84	4.42
JB Diego	0.57 (63.8)	6.70	4.26
Ketchum	0.20 (54.9)	7.12	3.93
Kipling	0.71 (67.1)	5.95	4.00
Lear	0.33 (58.1)	6.88	4.00
Malacca	0.65 (65.8)	7.64	4.94
Marksman	0.45 (61.0)	6.79	4.12
Mascot	0.49 (62.0)	7.61	4.72
Monty	0.33 (58.2)	7.05	4.09
Oakley	0.43 (60.6)	7.00	4.24
Panorama	0.30 (57.3)	7.45	4.23
Qplus	0.35 (58.8)	6.68	3.94
Robigus	0.76 (68.1)	6.33	4.23
Scout	0.97 (72.6)	7.02	5.00
Sherborne	0.46 (61.4)	7.30	4.44
Shogun	0.82 (69.5)	6.53	4.47
Soissons	0.52 (62.8)	7.44	4.65
Solstice	0.41 (60.1)	7.21	4.30
Viscount	0.37 (59.2)	7.34	4.32
Walpole	0.51 (62.5)	7.38	4.59
Welford	0.42 (60.4)	7.00	4.21
Xi19	0.31 (57.8)	6.68	3.85
Zebedee	0.80 (69.0)	7.26	5.00
d.f.	176	176	176
SED	0.254	0.353	0.468
F Probability	0.014	<.001	0.009
Grand mean	0.41 (59.9)	6.97	4.15

Table 6.22. Susceptibility of elite winter wheat varieties to take-all infection in a seedling pot test using B type *Ggt* isolates based on molecular classification.

Variety	Logit % roots infected with take-all (back-transformed means)	Mean no. roots/plant	Mean no. take-all infected roots/plant
Alchemy	-3.67 (2.5)	6.88	0.17
Avalon	-2.80 (5.8)	7.66	0.40
Bantam	-1.74 (14.9)	7.19	1.12
Battalion	-3.65 (2.5)	6.77	0.19
Brompton	-2.34 (8.8)	7.01	0.70
Cadenza	-2.59 (7.0)	7.08	0.62
Cassius	-3.90 (2.0)	6.49	0.14
Claire	-3.86 (2.1)	6.33	0.19
Conqueror	-1.23 (22.7)	6.69	1.50
Cordiale	-2.66 (6.6)	6.88	0.45
Duxford	-1.70 (15.5)	6.82	1.18
Edmunds	-0.87 (29.4)	7.50	2.18
Einstein	-1.26 (22.1)	6.12	1.48
Gallant	-3.96 (1.9)	7.77	0.16
Gladiator	-2.79 (5.8)	6.88	0.57
Grafton	-4.00 (1.8)	6.75	0.16
Hereford	-4.52 (1.1)	6.09	0.08
Hereward	-2.12 (10.7)	6.07	0.83
Humber	-1.82 (13.9)	6.87	0.99
Hyperion	-4.46 (1.1)	5.85	0.40
Invicta	-1.42 (19.5)	6.87	1.36
Istabraq	-4.15 (1.6)	6.48	0.10
JB Diego	-3.94 (1.9)	6.69	0.19
Ketchum	-2.68 (6.4)	7.34	0.46
Kipling	-3.06 (4.5)	6.68	0.30
Lear	-2.61 (6.8)	6.95	0.52
Malacca	-2.46 (7.8)	7.42	0.94
Marksman	-3.04 (4.6)	6.66	0.40
Mascot	-3.32 (3.5)	7.14	0.36
Monty	-1.51 (18.1)	7.38	1.48
Oakley	-3.09 (4.3)	7.24	0.48
Panorama	-3.02 (4.7)	7.16	0.35
Qplus	-1.84 (13.7)	6.74	0.96
Riband	-2.68 (6.4)	7.82	0.60
Robigus	-2.08 (11.1)	6.63	0.93
Scout	-3.99 (1.8)	7.37	0.20
Sherborne	-2.27 (9.4)	7.12	0.65
Shogun	-2.07 (11.2)	6.76	0.85
Soissons	-2.77 (5.9)	7.75	0.46
Solstice	-3.68 (2.5)	7.18	0.30
Viscount	-3.14 (4.2)	7.17	0.58

Table 6.22. Continued

Variety	Logit % roots infected with take-all (back-transformed means)	Mean no. roots/plant	Mean no. take-all infected roots/plant
Walpole	-3.48 (3.0)	7.35	0.22
Welford	-3.26 (3.7)	7.40	0.25
Xi19	-3.31 (3.5)	6.76	0.30
Zebedee	-2.58 (7.0)	7.23	0.62
d.f.	180	180	180
SED	0.644	0.326	0.272
F Probability	<.001	<.001	<.001
Grand mean	-2.83 (7.7)	6.96	0.61

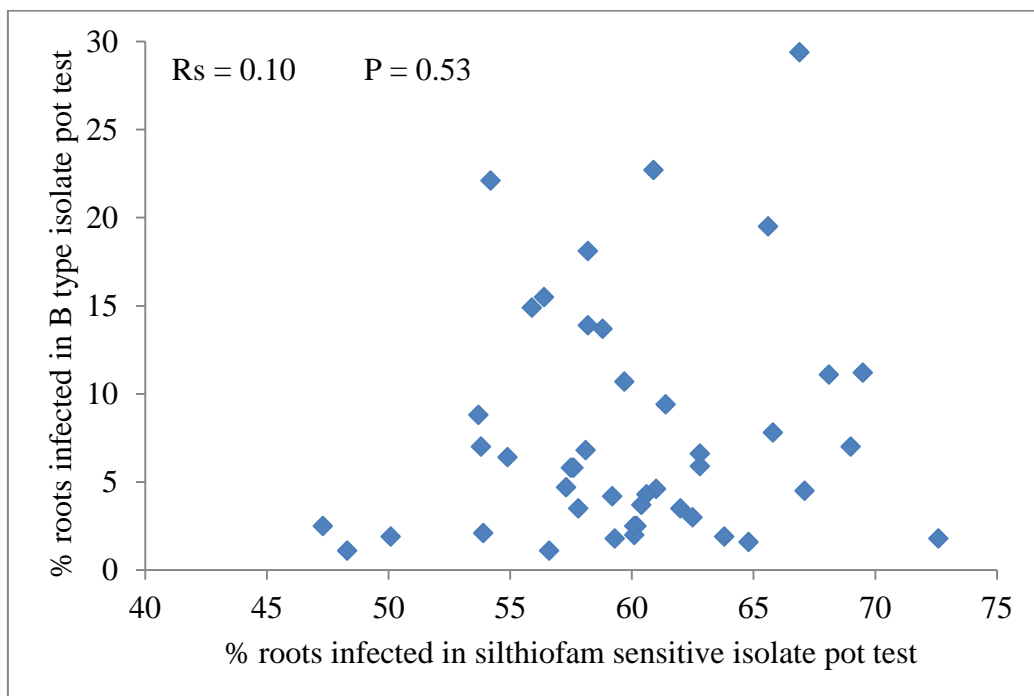


Figure 6.17. Correlation between the percentage roots infected of 44 elite winter wheat varieties in pot tests with silthiofam sensitive or B type *Ggt* isolates.

Table 6.23. Spearman’s rank correlation between susceptibility of wheat varieties in five week pot tests and susceptibility in the field.

Pot test	Field trial	Rs ¹	P	n ²
Silthiofam resistant	2009	0.05	0.76	44
Silthiofam resistant	2010	0.05	0.78	41
Silthiofam resistant	2011	0.72	0.04	8
Silthiofam sensitive	2009	0.00	0.98	44
Silthiofam sensitive	2010	-0.30	0.06	41
Silthiofam sensitive	2011	0.71	0.04	8
B type	2009	0.03	0.83	45
B type	2010	0.00	0.99	42
B type	2011	0.62	0.09	8

¹ Rs = Spearman’s rank correlation coefficient.

² n = Number of samples.

6.4. Discussion

Three years of field trials were carried out to assess the susceptibility of modern hexaploid wheat varieties to take-all. Take-all can be a very patchy disease in the field, and is highly influenced by environmental conditions, making it hard to detect treatment effects in the field. An alpha design with sub-blocking within main blocks was used in the 2009 and 2010 field trials, to help account for the potentially large background variation in these large 180 plot trials. The same alpha design was also used in the first wheat field trials used to evaluate the TAB trait (Chapter 3). Including the sub-blocking structure in analysis of the third wheat trials did not make a big difference to the residual variation and adjusted means, suggesting that take-all disease was quite uniformly distributed in these fields. By comparison including the sub-blocking structure in the first wheat trials, when take-all inoculum was building-up, had a bigger effect on the residual variation and adjusted means. Information in Hornby (1981) reports that visible patches of take-all were less obvious in a third wheat crop in Australia than during the first and second wheat crops, suggesting that inoculum distribution becomes more uniform during consecutive cereal cropping. The relatively uniform distribution in the third wheat trials in the PhD trials and extra sub-blocking structure created good conditions for discrimination between treatments if real differences were present.

In two of the three years (2009 and 2011) significant differences were detected between varieties in the level of take-all infection in the summer, but not in the spring. Take-all

intensity in 2009 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. In the lowest disease pressure year (2010) there were no significant differences between varieties in the summer. In both 2009 and 2011 the winter wheat variety Hereford stood out as the least susceptible variety (Hereford was not included in 2010 trial due to limited seed availability). In any individual year differences between varieties were either detected in the spring or summer, but not both. In all three field trial years there was no correlation between the amount of take-all disease in the spring and summer, suggesting that take-all disease development differs between varieties. In 2010 no significant differences were detected between varieties in the summer, but differences between varieties were found in the spring. In the combined year analysis differences in take-all susceptibility were significant in both the spring and summer, but again there was little correlation between the two sampling dates. Penrose (1995) also found that differences between varieties in the amount of take-all disease depended on sampling time (either tillering or anthesis in Penrose's study). In the combined year analysis Hereford and Kingdom had the lowest final disease severity in the summer. It should be noted however that Kingdom was only tested in one year of the trials (2011), so this result should be treated with caution. But this result suggests that Kingdom would be a good candidate for further trialling. Within the WGIN programme (www.wgin.org.uk) the Watkins world wheat collection (Miller et al., 2000) and Western European Gediflux wheat collection (Kolmer et al., 2008) are being screened for resistance to take-all in 3rd wheat field trials at Rothamsted (unpublished data). In a new pre-breeding LoLa project the best material identified within the WGIN programme has been bulked up for more detailed screening in replicated trials. Both Kingdom and Hereford are to be included in the 2013 trial.

To be useful to farmers and plant breeding programmes differences between varieties in take-all susceptibility should relate to higher yields and/or improvement in grain quality for the least susceptible varieties. In general take-all severity assessed during anthesis and grain filling in the summer is considered to relate best to yield (Hornby et al., 1998). Scott (1981) previously reported on the use of yield loss as a way of evaluating disease susceptibility between different host genotypes. In the PhD study the varieties in the third wheat trials were also sown in first wheat trials in the same years on the Rothamsted farm. The percentage yield loss could then be calculated between the varieties sown as first wheat crops in the absence of significant take-all and in the take-

all risk third wheat trials. In all three years there was no strong correlation between take-all severity and percentage yield losses. At face value, this finding may suggest that differences in susceptibility within modern wheat varieties would not be useful to improve 3rd wheat yields. However, the least susceptible variety Hereford had one of the highest yields in 2009 and 2011. Hereford was also high yielding in the first wheat trials so its percentage yield loss was similar to the other varieties. This could be because although Hereford has lower levels of take-all root infection it is less tolerant of the associated loss in root function. Secondly, yield losses in the third wheat field may be due to other factors, for example the nutrient and soil status differences between the first and third wheat fields. Wheat yields are known to be influenced by genotype x environment interactions even in the absence of disease. Ideally it would be better to have 1st wheat and 3rd wheat plots in the same field, to exclude potential variation in yields between fields. This method has been previously used by Jensen & Jorgensen (1976) to screen barley varieties for resistance to take-all. They prepared the field for the experiment by growing paired strips of either winter wheat or spring oats side by side for two years before testing material. They could then calculate relative grain yield of varieties with and without take-all.

In 2009 the above ground symptoms of take-all were recorded as a take-all patch score. This is the percentage of the plot area with prematurely ripened, stunted plants caused by take-all. For some varieties it was harder to score plots for take-all patches due to the timing of crop ripening for different varieties. Take-all patches are not as clearly visible as the crop progresses through ripening, meaning that scores for early ripening varieties may be lower as patches are less visible. In 2010 and 2011 take-all patches were not clearly visible in the trials so were not scored. In both of these years the low rainfall in the spring and summer (particularly in 2010) resulted in widespread premature ripening due to drought stress, which masked the symptoms of take-all. In the 2009 trial many of the varieties had quite low take-all patches compared with the take-all severity on the roots and low plot yields recorded. There were also relatively big differences between varieties in their take-all patch scores, but this was only quite weakly correlated with take-all severity and percentage yield loss. These results suggest there may be differences in the take-all 'tolerance' of elite winter wheat varieties to take-all. In Australia Penrose (1991) recorded the percentage of deadheads as an above ground symptom of take-all when evaluating different wheat genotypes for susceptibility to take-all. Similar to the take-all patch score in the PhD study the percentage of deadheads was not correlated to take-all infection or yield loss. Penrose (1991) found a

positive correlation between the percentage of deadheads and variety earliness in the Australian study, making measurement of deadheads at a single time point for all varieties not an appropriate measure for assessing susceptibility to take-all. Variety earliness was not recorded in the PhD study but difficulties assessing the above ground symptoms of take-all for different varieties in the 2009 trial suggest that the take-all patch score is also not an appropriate measure of take-all to screen wheat varieties.

Take-all tolerance, reflected in less above ground symptoms of take-all and/or less yield loss could be related to the ability of wheat varieties to compensate for loss of root function due to disease. Presumably varieties which take up water and nutrients more efficiently from the soil would be less affected by take-all. Or varieties which could increase water and nutrient uptake by non-diseased roots in response to loss of function in diseased roots. Drought resistant varieties could also potentially be more tolerant of take-all disease, as they are better able to cope with limited water uptake. The species barley has previously been reported as more tolerant of take-all because of its greater drought tolerance (Scott, 1981). Barley also typically reaches flowering two to three weeks before wheat, so is at a later growth stage by the time take-all is most severe (Gutteridge et al., 1993). This means that grain filling should usually be completed in barley before take-all disease is at its worst (Hornby et al., 1998). Disease escape in barley may also be helped by the production of large numbers of crown roots (Hornby et al., 1998). Previous investigations into the mechanisms contributing to different susceptibilities between species or varieties to take-all have looked at disease escape or tolerance by disease induced root production. Mattsson (1973) proposed that high crown root production could reduce the effects of take-all disease. The production of extra roots when attacked by *Ggt* is suggested as partially offsetting the loss of root function in already infected roots so that the plant can better tolerate infection. Varieties with inherently higher rooting densities may also be more tolerant of infection. Conversely high root numbers per plant may be a disadvantage earlier in the season as this has been shown to increase take-all infection as the probability of contact with the fungus increases (Colbach et al., 1997). Take-all is only able to colonise short distances in the soil by mycelial growth so that as root number/density increases take-all can spread more quickly.

Epidemiology studies were carried out to evaluate disease development during the season from March/April to harvest for a subset of wheat varieties in the trials. Different rates of take-all disease development could be potentially important as the growth and

yields of varieties with a slower rate of disease development earlier in the season would presumably be better. Whereas if severe disease develops early this could be particularly detrimental to yields (Gutteridge et al., 2003). Schoeny et al. (2001) have shown that accumulated disease incidence during the season from stem elongation to flowering can be related to yield losses. The final disease severity measured in the summer will not reflect these possible differences in disease development. The aim of the epidemiology studies were to assess disease development and also to identify potentially critical time periods during which differences between varieties occur. In epidemiology studies the use of different disease variables can alter the apparent patterns of disease development (Gilligan, 1994). Calculating the percentage of roots infected gives a measure of disease severity. However as total root production increases during the season this can create a decrease in the percentage of roots infected (Gilligan, 1994). In this study the number of take-all infected roots per plant was calculated to look at the absolute rate in increase in the numbers of roots infected. The total number of roots per plant was also estimated based on root counts of 10 plant sub samples per plot. This was to assess the total rooting densities of varieties. For both diseased and total root counts the roots were split up into the number of seminal and crown roots per plant. The seminal roots generally supply water and nutrients to the plant during the autumn while the crown roots become more important in the spring and summer. The effect of disease on yields will therefore depend on when and how many of the seminal and crown roots become infected (Bailey et al., 2005). The percentage of plants infected was also calculated as a measure of disease incidence.

In all three years there was no effect of variety on the average percentage of plants infected across the season from March/April to July. There was also no interaction of sampling date with variety, indicating that disease incidence does not differ between varieties. In 2009 and 2010 there were no differences in the absolute numbers of take-all infected seminal and crown roots per plant between varieties in the epidemiology study, or interactions with sampling date. In 2011, when the varieties Hereford and Hereward were sampled, there were close to significant interactions of sampling date with variety. In June there were more take-all infected seminal roots per plant for Hereward than Hereford. This may not be very important in terms of yield loss as in the summer the majority of water and nutrient uptake will be by the crown roots, not the seminal roots. However, in July there were also more take-all infected crown roots per plant for Hereward than Hereford. Differences in the absolute number of roots infected per plant suggest that Hereford has some way of restricting take-all development on the roots

later in the season, or that some other factor favours greater take-all development of the roots for Hereward. Rooting density could also influence the rate of disease development as a high rooting density increases the likelihood of secondary infections by root to root contact. The total number of crown roots per plant, estimated from subsamples, was greater for Hereford than Hereward, indicating that despite the increased probability of root to root contact Hereford is able to somehow restrict disease development. The higher number of roots for Hereford than Hereward may partly explain the very low take-all index of Hereford in the 2011 trial. Total root counts in the 2009 and 2010 epidemiology studies also showed differences in rooting densities between varieties, although no significant differences in the absolute number of take-all infected roots per plant. The varieties with highest and lowest total root numbers were not consistent between the two years, perhaps due to differences in environmental conditions between years and soil conditions between the different fields. By comparison to the rooting densities of varieties in the third wheat epidemiology study, plant samples were also taken from the same varieties in first wheat field trials. In 2009 there were 7-8 more roots on average per plant in July in the 1st wheat trial, in the absence of substantial take-all, compared with the heavily infected 3rd wheat trial. However, the effect of using different fields for the two trials cannot be eliminated as an influence on rooting ability. At the lower disease pressure years 2010 and 2011 there was no obvious difference between the 1st and 3rd wheat root counts.

A pot bioassay method, developed at Rothamsted to test the effect of fungicides on take-all, was used in this study to assess the susceptibility of wheat varieties to take-all at the seedling stage. In the pot tests mixtures of different sub populations of *Ggt* isolates were used. Significant differences between varieties in the percentage of roots infected with take-all were detected in two out of the three pot tests. However, the ranking of varieties was not similar between the two tests. There was not enough time to repeat the pot tests with the same mixtures of isolates so it is unclear whether the ranking of varieties is consistent and was due to the *Ggt* isolate mixtures used or perhaps due to differences in disease pressure between the tests. Two varieties Solstice and Xi19 had previously been identified by Richard Gutteridge as displaying some resistance to take-all in a limited number of seedling pot tests. In the PhD study tests neither of these two varieties displayed any obvious resistance to take-all compared with the other varieties. In the pot tests there were significant differences in total root numbers between varieties. Comparative root counts in the absence of take-all were not made so it is not possible to say whether the root counts in the pot tests were increased

by disease induced root production. In any case there were no significant correlations between the total number of roots per plant and the proportion of roots infected with take-all, indicating that there was no major effect of rooting ability at the seedling stage on take-all infection.

There were no significant correlations between the performance of varieties in the three pot tests and the take-all index of adult plants in the 2009 and 2010 field trials. In 2011, when there were only 8 varieties in common between the field and pot tests, there were significant correlations between seedling and adult plant susceptibility to take-all. However many different factors can influence disease in the field and when only eight varieties are studied, the positive correlations could be due to chance. With such small differences between these eight varieties in the pot tests, and no correlation of the seedling pot tests to the 2009 and 2010 field trials (when a larger number of varieties were compared), take-all susceptibility in the pot test cannot be considered to be a good predictor of field performance. This is at least the case for modern hexaploid wheat varieties, whose performance in the field is also not very consistent between sites and seasons. Where larger and more consistent differences in susceptibility to take-all exist between wheat genotypes or other species the pot test could still be a useful tool for screening genotypes. For example the moderately resistant species rye has been shown to display resistance to take-all at the seedling stage and in the field (Jorgensen & Jensen, 1970, Jensen & Joergensen, 1973, Linde-Laursen et al., 1973, Hollins et al., 1986, Hollins & Scott, 1990, Bithell et al., 2011a). But by comparison the wheat x rye hybrid triticale has often been very susceptible to take-all at the seedling stage, but moderately resistant in the field (Linde-Laursen et al., 1973, Hollins et al., 1986).

In summary this study has not identified varieties either highly resistant or highly tolerant to take-all. We have recently reported on differences between wheat varieties in their ability to build-up inoculum of the take-all fungus during a first wheat crop (McMillan et al., 2011), demonstrating a consistent genetic interaction between host genotype and the take-all fungus. In contrast to the build-up of inoculum in a first wheat crop the susceptibility of wheat varieties to take-all was not consistently expressed across sites and seasons in the third wheat field trials in this study. However, in individual field trial years and pot tests differences were detected in the susceptibility of wheat varieties to take-all. These results are in agreement with the large volume of literature investigating variation in resistance to take-all among cereal varieties (Nilsson, 1969, Scott, 1981, Scott et al., 1989). In this study one variety, Hereford, was

identified as showing some partial resistance to take-all in the proportion of roots infected in the summer (used to calculate the take-all index) and in the absolute number of roots infected in the 2011 epidemiology study. It is unclear whether this would be useful for plant breeding purposes and improvement of yields in 2nd and 3rd wheat crops as the lower level of disease was not strongly reflected in percentage yield loss between the 1st and 3rd wheat trials.

CHAPTER 7: EVALUATION OF OTHER WHEAT GERMPLASM FOR RESISTANCE TO TAKE-ALL

7.1. Introduction

One of the aims of this PhD was to identify sources of resistance to the take-all fungus. In Chapter 6 some differences were found between elite wheat varieties in their susceptibility to take-all, but there were no highly resistant varieties and there was little consistency between years. Historically, much of the work screening wheat varieties for resistance to take-all have taken place within hexaploid wheat varieties, but wild relatives of crop species have often been identified as good sources of resistance to a range of pathogens (Hajjar & Hodgkin, 2007). In this study the take-all susceptibility of the related species *Triticum monococcum* was explored in both pot and field tests. Diversity Array Technology (DART) marker analysis was utilised to assess whole genome diversity between *T. monococcum* accessions.

Triticum monococcum ($A^m A^m$), known as einkorn wheat, is a diploid wheat species that was domesticated from *T. boeoticum* and was widely cultivated during early cereal farming (Heun et al., 1997). After the Bronze Age the cultivation of higher yielding polyploid wheat species dominated and as a result *T. monococcum* varieties were left to grow in their natural habitats without intensive selection pressures from humans (Zohary & Hopf, 1993). *T. monococcum* is closely related to the main progenitor of the AA genome of tetraploid durum and hexaploid bread wheat, *T. urartu* (Huang et al., 2002). However, genetic research has shown that the *T. monococcum* $A^m A^m$ genome itself was not involved in the generation of modern durum and common wheat species (Dvorak et al., 1993). It has been suggested that *T. monococcum* may therefore contain high levels of genetic diversity that have not been exploited in modern wheat breeding programmes. Jing et al. (2007) reported that *T. monococcum* has many potentially useful traits that could be used in the genetic improvement of modern hexaploid wheat, including diversity in grain hardness, grain storage proteins, and germination under salt and drought stress.

Resistance to a range of pests and diseases have been reported in *T. monococcum*. This includes Russian wheat aphid (Deol et al., 1995), cereal aphids (Migui & Lamb, 2004, Radchenko, 2011), Hessian fly (Sharma et al., 1997), cereal cyst nematode (Singh et al., 2010), root lesion nematode (Sheedy et al., 2012), eyespot (Burt et al., 2010), fusarium head blight (Kopahnke et al., 2008), stem rust (Bai et al., 1998, Rouse & Jin, 2011), leaf

rust (Hussien et al., 1998), powdery mildew (Lebedeva & Peusha, 2006), septoria leaf blotch (Jing et al., 2008) and soil-borne cereal mosaic virus (Kanyuka et al., 2004).

The susceptibility of *T. monococcum* to take-all has previously been explored by a number of researchers but there are conflicting results between studies. Mielke (1974) reported that some *T. monococcum* lines were slightly less susceptible than hexaploid wheat species in greenhouse seedling tests. Although under field conditions all were severely attacked. Nilsson (1969) compiled a summary of the literature on the susceptibility of several hundred grass species to take-all. In this summary there were inconsistent results between studies with *T. monococcum* ranging from highly resistant to very susceptible. In recent years as part of the Wheat Genetic Improvement Network (WGIN) programme a small number of *T. monococcum* accessions have been assessed for susceptibility to septoria leaf blotch, fusarium head blight, cereal mosaic virus and eyespot (WGIN stakeholder newsletters, April 2006 and October 2007, www.wgin.org.uk). As part of the WGIN programme yearly field trials have been set up on the Rothamsted farm since 2006 to evaluate the susceptibility of *T. monococcum* to take-all. The first two trials (2006 and 2007) were carried out by Richard Gutteridge before the beginning of my PhD. The 2008 field trial was set up a year in advance of the beginning of this PhD (autumn 2007); after the samples were collected in summer 2008 these could be assessed for take-all infection once this PhD project had started. The *T. monococcum* accessions were tested for resistance against a number of control species: triticale, rye, oats and hexaploid bread wheat. As mentioned in Chapter 1, generally hexaploid wheat is very susceptible to take-all, rye is least susceptible and triticale is intermediate in susceptibility (Scott, 1981, Hollins et al., 1986, Mielke, 1992, Gutteridge et al., 1993). Oats is a non-host to take-all disease of wheat, *Ggt*, due to the production of the antifungal compound avenacin in its plant tissues (Scott et al., 1989). However, oats are susceptible to another variety of take-all caused by *Gaeumannomyces graminis* var. *avenae*. *Gga* produces the enzyme avenacinase which is able to convert avenacin to a less toxic form and so allow infection (Osborn et al., 1991). *Gga* is not commonly found in eastern Britain but does cause damage to oat crops in the northwest and is also able to infect wheat plants (Hornby et al., 1998). Oats was included as a control in the 2008 *T. monococcum* field trial to check that *Gga* is absent from the Rothamsted farm and that the take-all root disease in the trials is caused by *Ggt*, the take-all fungus of wheat. A small number of tetraploid wheat genotypes were included in the 2009 and 2010 trials to evaluate susceptibility to take-all at different ploidy levels.

The susceptibility of wheat genotypes in these trials was assessed by calculating the take-all index (Bateman et al., 2004) of adult plant samples taken from the field in June/July as in Chapter 6 where the susceptibility of elite winter wheat varieties to take-all was evaluated. Pot tests were also carried out to evaluate resistance at the seedling stage and compare with their field performance.

The genetic diversity of the *T. monococcum* accessions was assessed in this study using Diversity Array Technology (DArT). The DArT marker system was developed as a sequence-independent method for detecting DNA polymorphisms (Jaccoud et al., 2001). The presence or absence of specific DNA fragments is scored in representations of total genomic DNA from a population of organisms. Many loci are scored simultaneously making it a high throughput cost effective method for carrying out genetic fingerprinting. DArT markers have been developed for cereals such as triticale (Badea et al., 2011) and have already been successfully used in studies of genetic diversity in *Aegilops tauschii* (Sohail et al., 2012) and rye (Bolibok-Bragoszewska et al., 2009), association mapping in barley (Varshney et al., 2012), and genome mapping of tetraploid oats (Oliver et al., 2011). Diversity Array Technology is currently available or in development for 69 species, including a wide range of plant species as well as for two animal species (www.diversityarrays.com). Jing et al. (2009) first reported on the development of DArT markers for *Triticum monococcum*.

In this study the susceptibility to take-all of the model species *Brachypodium distachyon* was also investigated. In recent years *Brachypodium distachyon* has been proposed as a new model species to aid in the study of cereals and host-pathogen interactions (Draper et al., 2001, Garvin, 2007, Parker et al., 2008). Importantly the *Brachypodium* genus is closely related to all the key temperate cereal species. *Brachypodium distachyon* is considered an attractive option to help identify chromosomal regions of interest in cereals as it possesses a number of important attributes: small genome size (~ 160Mbp) with low amounts of repetitive DNA, chromosomal synteny with other cereal species, short lifecycle (less than 4 months) and a small physical size. A high-throughput transformation system has also been reported with the aim of developing *B. distachyon* as a model species for functional genomics studies (Pacurar et al., 2008).

Brachypodium distachyon is a host for a number of pathogens of temperate cereals including *Magnaporthe oryzae* (the rice blast fungus), *Puccinia striiformis* (wheat and barley yellow stripe rusts), and *Blumeria graminis* (powdery mildew). Recently Peraldi

et al. (2011) reported that *B. distachyon* would be a good model species for wheat to investigate infection by Fusarium head blight; *B. distachyon* was susceptible to both *Fusarium culmorum* and *Fusarium graminearum*, and the infection biology was similar to that in wheat. Differences in susceptibility of two *B. distachyon* lines to the *Fusarium* spp. were also found. The interaction of *Magnaporthe oryzae* with different *B. distachyon* ecotypes has also been investigated and infections found to closely follow that of *M. oryzae* in rice (Routledge et al., 2004). Resistant or susceptible responses to *M. oryzae* were found between *B. distachyon* ecotypes allowing the investigation of resistance mechanisms. To date, there is no published work on the susceptibility of *B. distachyon* ecotypes to the take-all fungus. The interaction of *Ggt* with *B. distachyon* was explored in pot tests within this PhD project to investigate whether it would be appropriate to use the *Ggt-B. distachyon* system to study resistance mechanisms to *Ggt*. The *B. distachyon* ecotypes used in this study included ABR 1, ABR 2 and ABR 3, previously shown to be susceptible to four different strains of *M. oryzae*; and ABR 5 and ABR 6, shown to be resistant to the same strains of *M. oryzae* (Routledge et al., 2004).

7.2. Materials and Methods

7.2.1. Diploid wheat (*T. monococcum*) field trials

Six field trials, in the harvest years of 2006, 2007, 2008, 2009, 2010 and 2011 were set up to evaluate the resistance of the diploid wheat *T. monococcum* to take-all disease. Standard procedures for evaluating resistance to take-all under field conditions are described in Chapter 2: General Materials and Methods. Fertiliser was applied according to standard Rothamsted farm practice. No growth regulator was applied and there were also no fungicide applications against foliar pathogens so that the susceptibility of the *T. monococcum* accessions to foliar diseases could be recorded if appropriate. The foliar disease data are not part of this study. In 2009 one dose each of the fungicides Unix® and Allure® were applied in error. Neither of these fungicides has any reported activity against *Ggt* so the trial was not compromised in terms of the take-all study. *T. monococcum* is very sensitive to herbicide application so a maximum of one dose of the herbicide Pacifica® was applied in the spring where required. In 2008 one dose each of the herbicides Arelon® 500 and Stomp® 400 SC were applied in error in the autumn. However the *T. monococcum* plots did not seem adversely affected by this one dose and showed good establishment in the spring.

In 2007 a severe blackgrass weed infestation in the spring and poor establishment of the *T. monococcum* plots meant that the trial had to be abandoned and was not fully sampled. Therefore only five years of data from the 2006, 2008, 2009, 2010 and 2011 field trials are reported here.

Over these five years of trials, 34 *T. monococcum* accessions were evaluated (Table 7.1). In the 2006 field trial 27 accessions were chosen for an initial screening. In 2008-2011 the *T. monococcum* accessions were selected based on extra information on their phenotypic and genetic diversity in other studies (Jing et al., 2007, Jing et al., 2008, Jing et al., 2009) and the results of the previous field trials and a limited number of take-all pot tests with some of the accessions (see section 7.2.2. for pot test procedures carried out). A selection of other hexaploid and tetraploid wheat varieties were tested in some years to evaluate wheat susceptibility to take-all at different ploidy levels (see Table 7.2 for variety treatments in each trial). The tetraploid wheat varieties evaluated in 2009 and 2010 were Alifen, Cham 1, Lahn, RWA 9 and RWA 10. The varieties Cham 1 and Lahn were of interest due to their adaptation to Mediterranean dryland conditions. Lahn (landrace Jennah Khetifa) is described as ‘moderately drought resistant’ and Cham 1 as having ‘high yield potential and yield stability’ (Nachit et al., 2001). Seed of the varieties RWA 9, RWA 10 and Alifen were received from Ms Lesley Smart, a colleague at Rothamsted. These varieties were considered to produce different levels of the metabolites 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA) in their root systems. In *in vitro* plate tests both these components had previously been reported to inhibit *Ggt* growth, but only when high concentrations had been used (Wilkes et al., 1999). In 2010 the wild goatgrass *Aegilops speltoides* (a progenitor species of tetraploid wheat) was also included in the field trial. Control species for comparison of take-all susceptibility included oats, rye and triticale in 2008 and rye and triticale in 2009, 2010 and 2011.

All trials were established in the autumn as third wheat field trials after two previous winter wheat crops for an expected high natural take-all disease pressure. Details of the field trials and sampling are given in Table 7.2. Trials were conducted in randomised block designs of five replicates of each genotype arranged in five fully randomised blocks, except that in 2008 there were two plots per block of three of the *T. monococcum* accessions (MDR037, MDR046 & MDR229). Field trial plans are given in Appendix 7.1. Plots measured 50 cm by 50 cm and 50-cm paths of bare soil were used to separate plots. Plots were hand sown with 60 seeds per plot over three rows. The

T. monococcum accessions were hand harvested to provide seed for the following year's experiment.

Plant samples (3 x 20 cm lengths of row per plot) were taken from each field trial at the beginning of July (GS 71-73) for take-all disease assessment as described in Chapter 2: General Materials and Methods. Data was statistically analysed by Rodger White. Take-all susceptibility of accessions was compared using analysis of variance in each individual year. A cross-season Residual Maximum Likelihood (REML) variance components analysis was used to analyse combined data from all years (excluding 2007 due to trial abandonment).

The 2006 field trial was sampled and assessed by R.J. Gutteridge before the PhD. The 2008 field trial was also set up and sampled in advance of the beginning of the PhD study. But plant samples were washed, dried and stored at room temperature to be assessed for take-all root infection once the PhD had commenced in October 2008.

Table 7.1. *T. monococcum* accessions used in the diploid wheat take-all resistance study field trials 2006 and 2008-2011¹.

Accession ²	Years in the trials	Species/Variety ⁴	Country of Origin	Year of Collection ⁴	Growth habit ⁴	Donor Centre ³
MDR001	1	<i>flavescens</i>	Algeria	ND	Spring	JIC
MDR002	5	<i>atriaristatum</i>	Balkans	ND	Spring	JIC
MDR025	1	<i>macedonicum; pseudoflavescens</i>	Ukraine	1923	Spring	VIR
MDR026	1	<i>pseudomacedonicum</i>	Ukraine	1923	Spring	VIR
MDR031	3	<i>monococcum; macedonicum</i>	Turkey	1927	Spring	VIR
MDR035	1	<i>flavescens; vulgare</i>	Austria	1930	Spring	VIR
MDR037	5	<i>macedonicum</i>	Armenia	1934	Spring	VIR
MDR040	1	<i>vulgare; macedonicum</i>	Bulgaria	1940	Spring	VIR
MDR043	3	<i>vulgare</i>	Greece	1950	Spring	VIR
MDR044	2	<i>hornemannii</i>	Turkey	1965	Spring	VIR
MDR045	1	<i>vulgare</i>	Denmark	1970	Spring	VIR
MDR046	4	<i>atriaristatum; macedonicum</i>	Romania	1970	Spring	VIR
MDR047	1	<i>macedonicum; vulgare</i>	Hungary	1970	Winter	VIR
MDR050	1	ND	Italy	ND	Spring	VIR
MDR217	4	1277	Turkey	ND	Spring	USDA
MDR218	4	2592	Turkey	ND	Spring	USDA
<u>MDR222</u>	1	3281	Turkey	ND	Spring	USDA
<u>MDR227</u>	1	Einkorn	United States	ND	Spring	USDA
<u>MDR228</u>	1	2497	Turkey	ND	Spring	USDA
MDR229	4	3962	Spain	ND	Spring	USDA
<u>MDR232</u>	3	<i>nigricultum</i>	Yugoslavia	ND	Winter	USDA
MDR236	1	I-1-1914	Hungary	ND	Spring	USDA
MDR243	1	2934	Romania	ND	Winter	USDA
<u>MDR244</u>	1	K930	Morocco	ND	Spring	USDA
MDR258	1	Einkorn	Israel	ND	Spring	USDA
<u>MDR261</u>	1	G2886	Iraq	ND	Spring	USDA

Table 7.1. Continued

Accession ²	Years in the trials	Species/Variety ⁴	Country of Origin	Year of Collection ⁴	Growth habit ⁴	Donor Centre ³
<u>MDR264</u>	1	G2900	Turkey	ND	Spring	USDA
<u>MDR279</u>	1	G2944	Turkey	ND	Spring	USDA
<u>MDR280</u>	4	G2946	Turkey	ND	Spring	USDA
<u>MDR286</u>	4	84TK154-034	Turkey	ND	Winter	USDA
MDR303	1	T-1600	Spain	ND	Spring	USDA
<u>MDR306</u>	1	957	Yugoslavia	ND	Spring	USDA
MDR308	5	DV92	Italy	ND	Spring	UC Davis
MDR650	3	PI355520	Iran	ND	ND	USDA

¹The 2007 trial was abandoned due to a severe weed infestation so information on the accessions tested in that year are not included in the table.

²*T. monococcum* accession information published in previous studies by colleagues at Rothamsted (Jing *et al.* 2007, 2008 and 2009) Bold and underlined accessions = not previously published.

³JIC = John Innes Centre, Norwich, UK; UC Davis = University of California, Davis, CA, USA; USDA = United States Department of Agriculture, Agricultural Research Service, Aberdeen, ID, USA; VIR = N.I. Vavilov Research Institute of Plant Industry, St Petersburg, Russia.

⁴ND = no data.

Table 7.2. Details of the diploid wheat and take-all resistance field trials 2006-2011.

Harvest year (field trial code)	Rothamsted field	Sowing date	Treatments	Date sampled	Growth stage (GS)
2006 (06/R/WW/615)	Delafield	06/10/05	27 <i>T. monococcum</i> accessions, 9 hexaploid wheat varieties	07/07/06	71-73
2007 ¹ (07/R/WW/710)	New Zealand	03/10/06	30 <i>T. monococcum</i> accessions, 6 hexaploid wheat varieties	Trial abandoned	
2008 (08/R/WW/810)	Long Hoos I&II	19/10/07	16 <i>T. monococcum</i> accessions, 3 control species ² , 14 hexaploid wheat varieties	01/07/08	71-73
2009 (09/R/WW/911)	Stackyard	20/10/08	5 <i>T. monococcum</i> accessions, 5 tetraploid wheat varieties, 2 control species ² , 10 hexaploid wheat varieties	09/07/09	71-73
2010 (10/R/WW/1034)	West Barnfield	28/10/09	13 <i>T. monococcum</i> accessions, 5 tetraploid wheat varieties, 2 control species ² , 11 hexaploid wheat varieties, 1 <i>Aegilops speltoides</i> accession	01/07/10	73
2011 (11/R/WW/1109)	Claycroft	29/10/10	12 <i>T. monococcum</i> accessions, 2 control species ² , 13 hexaploid wheat varieties	07/07/11	71-73

¹In 2007 a severe weed infestation meant the trial had to be abandoned.

²Control cereals species = oats, rye and triticale in 2008; rye and triticale in 2009, 2010 and 2011.

7.2.2. *T. monococcum* pot tests

Since 2006 a range of *T. monococcum* accessions have been evaluated for their susceptibility to take-all at the seedling stage in a five week pot test. The standard procedures for preparing inoculum, preparing soil, carrying out an inoculum-soil calibration and setting up the pot test are described in Chapter 2: General Materials and Methods. Percentage disease data from pot tests was always transformed using the logit transformation, to ensure equal variance, before further analysis. Transformed data was analysed using analysis of variance. Spearman's rank correlation was used to explore the relationships between different variates.

In July 2006 seven *T. monococcum* accessions (MDR002, MDR037, MDR040, MDR043, MDR044, MDR046 and MDR308) and six hexaploid wheat varieties (Avalon, Cordiale, Consort, Equinox, Florida and Hereward) were evaluated using the pot test method by my supervisor Richard Gutteridge. The soil used in the test was collected from the Rothamsted field 'Great Knott II' from fallow areas between plots. A mixture of 10 *Ggt* isolates was prepared as sand/maizemeal inoculum. The isolates used were 04.597.56.1, 04.597.23.5, 04.597.22.14, 04.597.22.10, 04.597.4.10, 04.597.56.3, 04.NFF.36.12, 04.NFF.36.9, 04.NFF.34.4 and 04.NFF.15.2. A dilution of 1:300 of this *Ggt* sand/maizemeal inoculum in silver sand was used in the test, with 50 g of this dilute inoculum being added to 300 g of the soil. Five replicates were set up per *T. monococcum* or hexaploid treatment.

Due to the abandonment of the 2007 field trial three pot tests were carried out instead in spring 2007 by Richard Gutteridge. Pot tests 1 and 3 screened 68 *T. monococcum* accessions (Table 7.3). In pot test 2 there was insufficient infection to compare the 48 *T. monococcum* accessions tested so these accession details and results are not included in this report. The winter wheat variety Hereward, and winter wheat variety Florida in pot test 1, were included as susceptible controls. The three pot tests were set up at weekly intervals on 07/02/07, 14/02/07 and 22/07/07. The experimental procedure was the same for all three tests. The soil used was collected from the Rothamsted field 'Meadow' from fallow areas between plots on the 11th August 2006. A mixture of 10 *Ggt* isolates was prepared as sand/maizemeal inoculum. The isolates used were the same as in the 2006 pot test above (04.597.56.1, 04.597.23.5, 04.597.22.14, 04.597.22.10, 04.597.4.10, 04.597.56.3, 04.NFF.36.12, 04.NFF.36.9, 04.NFF.34.4 and 04.NFF.15.2). A dilution of 1:250 *Ggt* sand/maizemeal inoculum in silver sand was used and 250 g of the prepared soil mixed with 50 g of the dilute inoculum.

Table 7.3. *T. monococcum* accessions screened in the 2007 diploid wheat pot tests 1 and 3.

Accession ¹	Species/Variety	Country of Origin ²	Year of Collection ²	Growth habit ²	Donor Centre ³
MDR001	<i>flavescens</i>	Algeria	ND	Spring	JIC
MDR002	<i>atriaristatum</i>	Balkans	ND	Spring	JIC
MDR024	<i>hornemannii; flavescens</i>	Russian Federation	1904	Spring	VIR
MDR025	<i>macedonicum; pseudoflavescens</i>	Ukraine	1923	Spring	VIR
MDR026	<i>pseudomacedonicum</i>	Ukraine	1923	Spring	VIR
MDR027	<i>monococcum, macedonicum</i>	Azerbaijan	1927	Spring	VIR
MDR028	<i>flavescens</i>	Germany	1927	Intermediate	VIR
MDR029	<i>flavescens</i>	Spain	1927	Spring	VIR
MDR030	<i>monococcum</i>	Spain	1927	Spring	VIR
MDR031	<i>monococcum; macedonicum</i>	Turkey	1927	Spring	VIR
MDR032	<i>vulgare</i>	Italy	1927	Spring	VIR
MDR033	<i>atriaristatum; vulgare</i>	Yugoslavia	1928	Spring	VIR
MDR034	<i>hornemannii; vulgare</i>	ND	1928	Spring	VIR
MDR035	<i>flavescens; vulgare</i>	Austria	1930	Spring	VIR
MDR036	<i>monococcum; pseudovulgare</i>	Czechoslovakia	1932	Spring	VIR
MDR037	<i>macedonicum</i>	Armenia	1934	Spring	VIR
MDR039	<i>hornemannii</i>	Georgia	1934	Spring	VIR
MDR040	<i>vulgare; macedonicum</i>	Bulgaria	1940	Spring	VIR
MDR041	<i>nigricultum; flavescens</i>	Albania	1950	Spring	VIR
MDR042	<i>flavescens; macedonicum</i>	ND	1950	Spring	VIR
MDR043	<i>vulgare</i>	Greece	1950	Spring	VIR
MDR044	<i>hornemannii</i>	Turkey	1965	Spring	VIR
MDR045	<i>vulgare</i>	Denmark	1970	Spring	VIR
MDR046	<i>atriaristatum; macedonicum</i>	Romania	1970	Spring	VIR
MDR047	<i>macedonicum; vulgare</i>	Hungary	1970	Winter	VIR
MDR048	<i>vulgare</i>	Sweden	ND	Spring	VIR

Table 7.3. Continued

Accession ¹	Species/Variety	Country of Origin ²	Year of Collection ²	Growth habit ²	Donor Centre ³
MDR049	<i>pseudohornemannii</i>	Iran	ND	Winter	VIR
<u>MDR214</u>	W49-64-2	United States	ND	Spring	USDA
<u>MDR215</u>	Metzger G68-3288	United States	ND	Spring	USDA
<u>MDR216</u>	1259	Turkey	ND	Spring	USDA
MDR217	1277	Turkey	ND	Spring	USDA
MDR218	2592	Turkey	ND	Spring	USDA
<u>MDR219</u>	2485	Turkey	ND	Spring	USDA
<u>MDR220</u>	3072	Turkey	ND	Spring	USDA
<u>MDR221</u>	3094	Turkey	ND	Spring	USDA
<u>MDR222</u>	3281	Turkey	ND	Spring	USDA
<u>MDR223</u>	3304	Turkey	ND	Spring	USDA
<u>MDR224</u>	3373	Turkey	ND	Spring	USDA
<u>MDR225</u>	3412	Turkey	ND	Spring	USDA
<u>MDR226</u>	3468	Turkey	ND	Spring	USDA
<u>MDR227</u>	Einkorn	United States	ND	Spring	USDA
<u>MDR228</u>	2497	Turkey	ND	Spring	USDA
MDR229	3962	Spain	ND	Spring	USDA
<u>MDR230</u>	<i>flavescens</i>	Ethiopia	ND	Spring	USDA
<u>MDR231</u>	<i>laetissimum</i>	Ethiopia	ND	Spring	USDA
<u>MDR232</u>	<i>nigricultum</i>	Yugoslavia	ND	Winter	USDA
<u>MDR233</u>	<i>kaplouras</i>	Greece	ND	Spring	USDA
MDR234	Einkorn	Kenya	ND	Spring	USDA
<u>MDR289</u>	TU85-056-04	Turkey	ND	ND	USDA
<u>MDR290</u>	TU85-081-03	Turkey	ND	Winter	USDA
<u>MDR291</u>	TU85-082-02-1	Turkey	ND	ND	USDA

Table 7.3. Continued

Accession ¹	Species/Variety	Country of Origin ²	Year of Collection ²	Growth habit ²	Donor Centre ³
<u>MDR292</u>	TU85-082-02-2	Turkey	ND	ND	USDA
<u>MDR293</u>	TU85-082-02-04	Turkey	ND	Winter	USDA
<u>MDR294</u>	84TK256-011	Turkey	ND	Spring	USDA
<u>MDR295</u>	84TK261-001	Turkey	ND	Winter	USDA
<u>MDR296</u>	84TK299-001	Turkey	ND	Winter	USDA
<u>MDR297</u>	84TK302-002	Turkey	ND	Winter	USDA
<u>MDR298</u>	84TK329-006	Turkey	ND	Winter	USDA
<u>MDR299</u>	84TK330-003	Turkey	ND	Winter	USDA
<u>MDR300</u>	84TK331-005	Turkey	ND	Winter	USDA
<u>MDR301</u>	030689-0303	Turkey	ND	Winter	USDA
<u>MDR302</u>	290	Russian Federation	ND	Spring	USDA
MDR303	T-1600	Spain	ND	Spring	USDA
<u>MDR304</u>	982	Yugoslavia	ND	Spring	USDA
<u>MDR305</u>	954	Yugoslavia	ND	Spring	USDA
<u>MDR306</u>	957	Yugoslavia	ND	Spring	USDA
<u>MDR307</u>	959	Yugoslavia	ND	Spring	USDA
MDR308	DV92	Italy	ND	Spring	UC Davis

¹ *T. monococcum* accession information published in previous studies by colleagues at Rothamsted (Jing *et al.* 2007, 2008 and 2009) Bold and underlined accessions = not previously published.

² ND = no data.

³ JIC, John Innes Centre, Norwich, UK; UC Davis = University of California, Davis, CA, USA; USDA = United States Department of Agriculture, Agricultural Research Service, Aberdeen, ID, USA; VIR, NI Vavilov Research Institute of Plant Industry, St Petersburg, Russia.

During my PhD I evaluated the sixteen *T. monococcum* accessions in the 2008-2011 field trials using the pot test method in spring 2012. Rye and triticale were also included as treatments to compare the seedling test with their known resistances to take-all in the field as adult plants (rye is quite resistant and triticale is moderately susceptible; see introduction to this chapter for information on species controls). Soil was collected in summer 2009 from fallow areas in the Rothamsted field 'Great Field IV'. A mixture of 14 *Ggt* isolates (BC02, BC03, BC04, BC05, BC10, BC15, BC16, BC17, BC19, BC23, BC24, BC26, BC28 and BC34) were used in the test as representative of a field population to include isolates characterised as A or B type and sensitive or resistant to silthiofam in the molecular and fungicide classification tests in Chapter 5: Characterisation of a new *Ggt* isolate collection. Due to a low level of infection on Hereward plants in the soil calibration the protocol was modified to mix 150 g soil with 100 g damp sand before adding 50 g of a 1:50 dilution of artificial sand/maizemeal *Ggt* inoculum with silver sand. Five replicates were set up per accession and placed in a controlled environment room (16 hour day, 70% RH, 15°C day/10°C night, twice weekly watering) for five weeks before take-all disease assessment.

7.2.3. *T. monococcum* DArT diversity analysis

DArT marker assays were carried out by Triticarte, Australia (www.triticarte.com.au). Twenty *T. monococcum* accessions were genotyped in an array using 1041 markers. Colleagues at Rothamsted obtained contrasting results in aphid feeding tests with different sources of MDR037 seed. Three samples of MDR037, originating from different seed stocks, were therefore analysed. Accessions were scored at each marker for the presence or absence of the DNA fragment of interest, represented by a '1' or '0'. If a marker could not be reliably scored for a particular sample this was treated as missing datum and scored as '-'. A Jaccard similarity matrix was generated and used to carry out a principal coordinate analysis in Genstat (Payne et al., 2009). A hierarchical cluster analysis was also carried out based on the generated Jaccard similarity matrix.

7.2.4. *B. distachyon*-take-all pathogenicity test

At the beginning of the PhD during *Ggt* isolate collection and characterisation the susceptibility of five *B. distachyon* ecotypes (Table 7.4) to take-all was assessed in a pathogenicity assay. Ten *Ggt* isolates (5 silthiofam resistant isolates, BC04, BC05, BC16, BC23 and BC26; and 5 silthiofam sensitive isolates, BC03, BC06, BC10, BC12

and BC14) were tested to assess their ability to cause disease. Each of the five sensitive isolates and each of the five resistant isolates were treated as replicates within the design as either sensitive or resistant in 5 randomised blocks. Each block contained 3 pots of each *Brachypodium* ecotype with a sensitive isolate layer of inoculum, a resistant isolate layer of inoculum and a control non-colonised agar layer within the pot, respectively (15 pots in total per block). Plastic pots (6 cm diameter by 10 cm deep) were filled with 120 cm³ moist sand. *Ggt* colonised agar ($\frac{1}{3}$ Petri dish for each pot) was cut up and mixed with 50 cm³ sand and this mixture added as a layer of inoculum on top of moist sand in the plastic pots. Control pots contained non-colonised PDA. The pots were then topped up with a further 80 cm³ moist sand over the layer of inoculum. Five *Brachypodium* seeds were placed on the sand and covered with coarse horticultural grit. Pots were placed in a controlled environment room (16 hour day, 70% RH, 21°C day/16°C night, twice weekly watering) for 5 weeks. Plants were then removed and the roots washed. Roots were assessed for take-all infection and the number of plants and roots infected with take-all were recorded.

Table 7.4. *Brachypodium distachyon* ecotypes¹ used in take-all pathogenicity study.

Accession no.	Ecotype	Country of origin
MDR672	ABR 1	Turkey
MDR673	ABR 2	France
MDR674	ABR 3	Spain
MDR675	ABR 5	Spain
MDR676	ABR 6	Spain

¹ All accessions were obtained from IBERS, Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Wales.

7.3. Results

7.3.1. Response of *T. monococcum* to take-all under field conditions

The take-all index, calculated by grading whole plant systems from plot samples by the proportion of roots affected by take-all into categories (slight 1 to severe), assesses both the incidence and severity of take-all. In all five individual field trial years there were statistically significant differences in the take-all index score of the genotypes tested (Tables 7.5a and 7.5b). The mean take-all index varies considerably from year to year, with a mean take-all index between 30 and 50 in the 2006, 2008 and 2009 trial years and a take-all index of less than 15 in 2010 and 2011. In general the hexaploid wheat varieties displayed relatively high take-all indexes, reflecting the known high susceptibility to take-all of modern wheats (Table 7.5b). The control cereal species, used to benchmark the response of the *T. monococcum* accessions, also performed as expected. Oats, as a non-host to *Ggt*, in the 2008 field trial exhibited no take-all infection. Rye, as a much less susceptible cereal species compared with hexaploid wheat, showed the lowest take-all index out of all the genotypes tested in the 2008-2011 field trials. The wheat x rye hybrid cereal species triticale showed an intermediate level of susceptibility between the hexaploid wheat varieties and the rye control.

The 2006 field trial, used as an initial screen of 27 different *T. monococcum* accessions with 9 hexaploid wheat varieties, revealed a range of susceptibilities to take-all within the diploid wheat species. The mean take-all index over the trial was 49.1, reflecting a relatively high take-all disease pressure in this year. Under these conditions the majority of accessions had comparable take-all indexes to the hexaploid wheats but there was also evidence of potential partial resistance to take-all in some accessions (Take-all index under 30: MDR279 and MDR286).

Some of the *T. monococcum* accessions were retested in the 2008-2011 field trials and new *T. monococcum* accessions included based on seed availability, information on their genetic diversity and results from seedling pot tests (see section 7.3.2. for 2006 and 2007 pot test results). Two *T. monococcum* accessions, MDR031 and MDR046, stood out as consistently showing the lowest susceptibility to take-all over multiple years of trialling, having take-all indexes intermediate between that of the control species rye and triticale (Table 7.5a). MDR286, first identified as showing evidence of potential partial resistance to take-all in the 2006 field trial, also shows low levels of take-all

infection in the 2008, 2010 and 2011 field trials (MDR286 was not included in 2009 field trial). In contrast MDR002, MDR043 and MDR308 were consistently very susceptible to take-all infection.

In 2009 and 2010 five tetraploid wheat genotypes were evaluated for their susceptibility to take-all. In both years all five genotypes showed very high susceptibility to take-all (Table 7.5a). This is particularly noticeable in 2010, where despite the overall low take-all disease pressure (mean TAI over trial: 13.7), the five tetraploid genotypes had take-all indexes ranging from 29-42. In contrast the hexaploid wheat genotypes (considered to be fully susceptible to take-all) had take-all indexes ranging from only 5.4-13.3. In 2010 the wild goatgrass *Aegilops speltoides* (a progenitor species of tetraploid wheat) was also included in the field trial. This species exhibited an intermediate level of take-all susceptibility (TAI 21.8) between the hexaploid wheat varieties and tetraploid genotypes.

Table 7.5a. Take-all index of control cereal species, *T. monococcum* accessions, tetraploid wheat varieties and *Ae. speltoides* in the diploid wheat and take-all resistance field trials 2006-2011.

Treatment	Take-all Index (0-100)				
	Year				
	2006 ¹	2008 ²	2009	2010	2011
Control species					
Oats		0.0			
Rye		5.5	3.2	1.1	0.8
Triticale		23.3	35.6	5.9	3.0
Hereward (hexaploid wheat)	44.3	54.7	59.0	11.0	12.9
<i>T. monococcum</i> accessions					
MDR001	69.0				
MDR002	54.9	31.2	62.0	13.3	7.7
MDR025		35.2			
MDR026		32.4			
MDR031		7.6		4.5	0.9
MDR035	53.5				
MDR037	41.2	29.9	60.6	12.3	5.6
MDR040	69.8				
MDR043	70.4	43.8		19.4	
MDR044	52.6	25.9			
MDR045	50.8				
MDR046		12.3	26.7	4.1	1.4

Table 7.5a. Continued

Treatment	2006 ¹	2008 ²	2009	2010	2011
<i>T. monococcum</i> accessions (continued)					
MDR047	45.1				
MDR050	55.4				
MDR217	33.9	21.6		7.1	3.9
MDR218	34.0	17.1		10.7	3.4
MDR222	39.7				
MDR227	57.9				
MDR228	52.1				
MDR229		18.4	37.6	11.0	7.2
MDR232		17.3		4.7	3.5
MDR236	42.0				
MDR243	42.8				
MDR244	52.8				
MDR258	66.8				
MDR261	66.8				
MDR264	42.4				
MDR279	28.3				
MDR280	37.0	20.0		12.5	14.4
MDR286	22.5	17.6		6.3	3.4
MDR303	66.1				
MDR306	50.0				
MDR308	46.3	28.9	73.1	15.4	4.7
MDR650 (PI355520)		20.4		5.2	5.6
Tetraploid wheat varieties					
Alifen			75.0	33.5	
Cham 1			61.6	36.8	
Lahn			78.7	29.3	
RWA 9			82.1	40.4	
RWA 10			64.2	42.0	
Additional species					
<i>Aegilops speltoides</i>				21.8	
d.f.	140	143	84	124	104
SED	11.8		8.9	4.9	3.5
min.rep		9.9			
max-min		8.6			
max.rep		7.0			
F Probability ³	0.002	<0.001	<0.001	<0.001	0.048
Grand Mean	49.1	30.3	50.9	13.7	5.2

¹ 2006 field trial data generated by Richard Gutteridge before commencement of the PhD study.

² In 2008 there were five replicates per genotype, except for 10 replicates of the *T. monococcum* accessions MDR037, MDR046, MDR229.

³ All d.f., SED and F Probability values refer to analysis of all of the *T. monococcum*, tetraploid and hexaploid varieties in any one year. See table 7.5b to compare *T. monococcum* and tetraploid variety results with hexaploid TAI values.

Table 7.5b. Take-all index of control cereal species and hexaploid wheat varieties in the diploid wheat and take-all resistance field trials 2006-2011.

Treatment	Take-all Index (0-100)				
	Year				
	2006 ¹	2008 ²	2009	2010	2011
Control species					
Oats		0.0			
Rye		5.5	3.2	1.1	0.8
Triticale		23.3	35.6	5.9	3.0
Hereward (hexaploid wheat)	44.3	54.7	59.0	11.0	12.9
Hexaploid wheat varieties					
Alchemy		44.0			2.4
Bantam		32.6			4.2
Battalion			46.3		
Bob White	40.2	38.6			
Cassius		45.5			4.2
Chinese Spring		62.6			
Claire	50.6				
Consort	36.2				
Cordiale	48.8	43.2	35.7	7.3	
Duxford			46.5	7.5	5.3
Einstein		30.9	47.7	13.3	
Equinox	63.0				
Gallant				10.0	
Hereford					4.2
Hyperion		39.8			
Invicta					5.4
Istabraq	43.0		45.6		8.4
JB Diego			49.1		
Lear		45.1			3.4
Napier	50.0				
Panorama		44.4			
Paragon			39.6	8.7	
Q Plus		46.3			
Robigus	46.8	48.8	57.4	11.0	8.4

Table 7.5b. Continued

Treatment	2006 ¹	2008 ²	2009	2010	2011
Hexaploid wheat varieties (continued)					
Shogun					5.2
Solstice			33.3	5.4	3.4
Tybalt				7.2	
Welford				11.1	
Xi19				8.6	6.7
Zebedee		44.9			
d.f.	140	143	84	124	104
SED	11.8		8.9	4.9	3.5
min.rep		9.9			
max-min		8.6			
max.rep		7.0			
F Probability ³	0.002	<0.001	<0.001	<0.001	0.048
Grand Mean	49.1	30.3	50.9	13.7	5.2

¹ 2006 field trial data generated by Richard Gutteridge before commencement of the PhD study.

² In 2008 there were five replicates per genotype, except for 10 replicates of the *T. monococcum* accessions MDR037, MDR046, MDR229.

³ All d.f., SED and F Probability values refer to analysis of all of the *T. monococcum*, tetraploid and hexaploid varieties in any one year. See table 7.5a to compare hexaploid results with *T. monococcum* and tetraploid variety TAI values.

When a combined year analysis was carried out using REML there were highly significant differences in take-all susceptibility between genotypes (Table 7.6, $P < 0.001$). REML combines information from all five field experiments and provides estimates of treatment effects. Many of the genotypes are not well represented from year to year; only five treatments out of all seventy four tested genotypes are present in all five years (MDR002, MDR037, MDR308 Hereward and Robigus). This makes it less reliable to combine information across years. It is perhaps more useful to look at the analyses of treatment effects in individual years as above. In the combined analysis the take-all index of control species rye and triticale was not very different, 16.32 and 21.97 respectively. Triticale was also closer to rye in susceptibility than to the hexaploid wheat control variety Hereward. Over all years MDR279, MDR031 and MDR046 were least susceptible out of the 34 *T. monococcum* accessions. However MDR279 was only in one year of the field trials (2006) so it is not possible to say how consistently resistant

this accession is. MDR031 and MDR046 were included in 3 and 4 years of field trials respectively. In these trials these two accessions were consistently the least susceptible to take-all and intermediate in resistance to rye and triticale. In contrast MDR002, MDR043 and MDR308 (some of the most susceptible accessions in individual years), were intermediate in susceptibility in the combined year analysis. MDR040 (only included in 2006 trial) was the most susceptible accession in the combined year analysis. The thirty hexaploid wheat varieties had take-all indexes of 21.04 to 60.03. However, many of the hexaploid wheat varieties were only included in one or two years out of the five trials. The *T. monococcum* accessions take-all indexes ranged from 13.09 to 54.60, showing a similar range of susceptibilities as the hexaploid wheat varieties. As in the 2009 and 2010 individual field trial results the tetraploid wheat varieties all had relatively high take-all indexes in the combined year analysis.

Table 7.6. Combined year analysis of the take-all index of *T. monococcum* accessions, hexaploid and tetraploid wheat varieties in five years of field experiments (2006, 2008-2011).

Treatment	Take-all Index (0-100)
Control species	
Oats	-2.31
Rye	16.32
Triticale	21.97
Hereward (hexaploid wheat)	32.24
<i>T. monococcum</i> accessions	
MDR279	13.09
MDR046	18.90
MDR031	19.65
MDR232	21.84
MDR280	22.49
MDR217	22.98
MDR650 (PI355520)	23.49
MDR218	23.60
MDR222	24.51
MDR229	25.25
MDR236	26.88
MDR264	27.22
MDR037	27.41
MDR243	27.60
MDR308	28.69
MDR002	29.68

Table 7.6. Continued

Treatment	Take-all Index (0-100)
<i>T. monococcum</i> accessions (continued)	
MDR286	29.77
MDR047	29.96
MDR026	30.06
MDR044	30.13
MDR025	32.91
MDR306	34.86
MDR045	35.60
MDR228	36.98
MDR244	37.62
MDR035	38.31
MDR043	38.96
MDR050	40.29
MDR227	42.79
MDR303	50.98
MDR261	51.63
MDR258	51.67
MDR001	53.85
MDR040	54.60
Tetraploid wheat varieties	
Lahn	47.81
Alifen	50.96
Cham 1	51.88
RWA 10	56.33
RWA 9	58.01
Additional species	
<i>Aegilops speltoides</i>	38.27
Hexaploid wheat varieties	
Consort	21.04
Solstice	21.96
Tybalt	23.68
Hereford	23.69
Alchemy	23.78
Bantam	24.28
Shogun	24.71
Duxford	24.74
Paragon	24.74
Lear	24.78
Invicta	24.89
Cassius	25.51
Xi19	25.80
Cordiale	26.09

Table 7.6. Continued	
Treatment	Take-all Index (0-100)
Hexaploid wheat varieties (continued)	
Gallant	26.45
Welford	27.63
Istabraq	27.72
Battalion	28.81
Einstein	29.39
Robigus	29.57
Bob White	31.02
JB Diego	31.81
Napier	34.83
Claire	35.44
Hyperion	37.49
Panorama	42.12
Zebedee	42.54
Q Plus	44.02
Equinox	47.83
Chinese Spring	60.33
d.f.	73
SED (Average)	7.81
Wald statistic	547.5
F Probability	<0.001
Grand mean	32.33

7.3.2. Response of *T. monococcum* to take-all in pot tests

Hereward seedlings in control pots without addition of *Ggt* sand/maizemeal inoculum were free from take-all in all of the pot tests.

In 2006 the pot test of seven *T. monococcum* accessions and six hexaploid wheat varieties revealed that there were statistically significant differences in the percentage of roots infected between the different accessions tested at the seedling stage (Table 7.7). In agreement with field results it demonstrates a range of susceptibilities of the *T. monococcum* species to take-all, with six out of the seven accessions showing similar infection levels to the susceptible hexaploid wheats. In this test MDR046 shows a reduction in take-all infection compared with the other accessions. MDR046 was not screened in the 2006 field trial but based on this pot test result MDR046 was then included in the 2008-2011 field trials and consistently displayed some partial resistance

to take-all as adult plants in the field in all four of these trials (see section above 7.3.1). This partial resistance was more pronounced in the field than at the seedling stage.

Table 7.7. Susceptibility of *T. monococcum* and modern hexaploid wheat varieties to take-all infection in a seedling pot test 2006¹.

Treatment	Logit percentage roots infected with take-all (back transformed means)
<i>T. monococcum</i> accessions	
MDR046	-0.46 (28.2)
MDR308	-0.27 (36.2)
MDR037	-0.17 (41.0)
MDR044	-0.16 (41.6)
MDR002	-0.04 (47.4)
MDR040	0.01 (50.1)
MDR043	0.06 (52.5)
Hexaploid wheat variety	
Florida	-0.23 (38.0)
Cordiale	-0.22 (38.9)
Equinox	-0.15 (42.2)
Avalon	-0.14 (42.6)
Consort	-0.10 (44.4)
Hereward	-0.01 (48.8)
d.f.	48
SED (logits)	0.100
F Probability	<.001
Grand mean	-0.145 (42.5)

¹ Data generated by Richard Gutteridge before the PhD study commenced.

In 2007 three pot tests were carried out by Richard Gutteridge on a total of 116 *T. monococcum* accessions. There was insufficient disease in the second pot test so the 48 *T. monococcum* accessions in this test could not be evaluated at the seedling stage.

In pot test 1 the mean percentage roots infected on 47 *T. monococcum* accessions ranged from 11.7% to 52.4%, and there was statistically significant differences between accessions ($P < .001$; Table 7.8). In comparison the fully susceptible hexaploid wheat varieties Hereward and Florida had 46.8% and 33.1% take-all infected roots respectively. The average number of roots per plant varied with accession ($P < .001$) but this was not related with the percentage roots infected with take-all (Spearman rank [Rs] = -0.20, $P = 0.16$, $n = 47$). There was a strong positive relationship between the number of take-all infected roots per plant and the percentage roots infected ($R_s = 0.87$, $P < .001$, $n = 47$). This suggests that the resistance mechanism by which some accessions have

low amounts of take-all infection is not a result of extra rooting ability to compensate for the roots infected but the result of restricting the take-all fungus from infecting. There were 6 *T. monococcum* accessions with significantly less percentage roots infected than the hexaploid wheat variety Florida (MDR229, MDR046, MDR232, MDR025, MDR026 and MDR031). In the field trials both MDR046 and MDR031 displayed consistently the lowest levels of take-all root infection and MDR229 and MDR232 have also shown lower levels of susceptibility to take-all (Table 7.5a). However MDR025 and MDR026 were two of the most susceptible accessions in the 2008 field trial revealing that screening at the seedling stage is not always representative of performance as mature plants in the field.

Table 7.8. Susceptibility of *T. monococcum* to take-all infection in seedling pot test 1 2007¹.

Treatment	Logit percentage roots infected with take-all (back transformed means)	Total number of roots/plant	Number of take-all infected roots/plant
<i>T.monococcum</i> accessions			
MDR229	-0.99 (11.7)	5.696	0.696
MDR046	-0.72 (18.8)	5.625	1.055
MDR232	-0.69 (19.7)	6.155	1.264
MDR025	-0.64 (21.2)	5.232	1.114
MDR026	-0.63 (21.7)	5.791	1.280
MDR031	-0.62 (22.1)	5.122	1.160
MDR228	-0.50 (26.5)	5.220	1.420
MDR217	-0.48 (27.3)	5.768	1.596
MDR221	-0.46 (28.1)	5.524	1.567
MDR030	-0.45 (28.4)	6.460	1.900
MDR219	-0.43 (29.3)	5.300	1.580
MDR027	-0.42 (29.6)	5.460	1.633
MDR028	-0.41 (29.9)	5.366	1.629
MDR044	-0.38 (31.2)	5.420	1.720
MDR224	-0.38 (31.5)	4.922	1.604
MDR225	-0.37 (31.9)	5.280	1.700
MDR001	-0.36 (32.1)	5.856	1.913
MDR024	-0.36 (32.1)	5.300	1.720
MDR215	-0.36 (32.4)	6.025	1.984
MDR045	-0.35 (32.7)	4.938	1.647
MDR231	-0.35 (32.8)	5.250	1.760
MDR227	-0.34 (32.2)	5.780	1.940

Table 7.8. Continued

Treatment	Logit percentage roots infected with take-all (back transformed means)	Number of roots/plant	Number of take-all infected roots/plant
<i>T. monococcum</i> accessions (continued)			
MDR002	-0.34 (33.4)	5.360	1.820
MDR223	-0.33 (33.5)	5.409	1.831
MDR222	-0.33 (33.8)	5.038	1.729
MDR220	-0.32 (33.8)	5.053	1.718
MDR048	-0.32 (34.0)	5.251	1.813
MDR043	-0.31 (34.3)	4.844	1.693
MDR218	-0.31 (34.6)	5.440	1.900
MDR047	-0.31 (34.6)	5.640	1.980
MDR216	-0.30 (34.8)	5.784	2.022
MDR234	-0.30 (34.9)	5.160	1.820
MDR036	-0.29 (35.6)	4.467	1.613
MDR033	-0.28 (35.9)	6.152	2.217
MDR039	-0.26 (36.6)	5.420	2.000
MDR233	-0.25 (37.1)	5.420	2.040
MDR032	-0.23 (38.0)	5.125	1.975
MDR041	-0.22 (38.6)	5.625	2.210
MDR226	-0.22 (38.8)	5.220	2.061
MDR034	-0.22 (38.8)	5.193	2.058
MDR037	-0.21 (39.0)	5.032	1.992
MDR230	-0.20 (39.7)	5.620	2.260
MDR040	-0.19 (40.0)	5.480	2.220
MDR214	-0.16 (41.5)	5.360	2.240
MDR029	-0.16 (41.6)	7.158	3.010
MDR042	-0.03 (48.3)	4.540	2.220
MDR035	0.06 (52.4)	4.360	2.300
Hexaploid wheat variety			
Florida	-0.34 (33.1)	7.337	2.508
Hereward	-0.05 (46.8)	5.905	2.790
d.f.	197	197	197
SED ²			
min.rep	0.100	0.233	0.249
max-min	0.087	0.202	0.216
F Probability	<.001	<.001	<.001
Grand mean	-0.34 (33.2)	5.476	1.854

¹ Data generated by Richard Gutteridge before the PhD study commenced.

² Five replicates per *T. monococcum* accession and Florida, ten replicates of Hereward.

In pot test 3 there was a lower overall amount of take-all infection, with 25.5% roots infected on the take-all susceptible control variety Hereward. The mean percentage roots infected on the 21 *T. monococcum* accessions evaluated ranged from 8.4% to 31.4% and again there were statistically significant differences between accessions ($P < .001$; Table 7.9). As in pot test 1 the accessions in pot test 3 had significantly different average numbers of roots per plant ($P < .001$) but this was not related to the disease measure percentage roots infected with take-all ($R_s = 0.15$, $P = 0.49$, $n = 21$). There were 6 *T. monococcum* accessions with less than 15% roots infected in this pot test (MDR306, MDR302, MDR300, MDR289, MDR297 and MDR298). Of these 6 accessions only MDR306 has been tested in the field in the 2006 field trial. MDR306 did not show any evidence of resistance to take-all in this trial supporting the view that a combination of pot and field trials is necessary to fully evaluate wheat germplasm for susceptibility to take-all.

Table 7.9. Susceptibility of *T. monococcum* to take-all infection in seedling pot test 3 2007¹.

Treatment	Logit percentage roots infected with take-all (back transformed means)	Number of roots/plant	Number of take-all infected roots/plant
<i>T. monococcum</i> accessions			
MDR306	-1.16 (8.4)	4.351	0.427
MDR302	-1.02 (11.0)	4.928	0.621
MDR300	-0.92 (13.1)	3.933	0.417
MDR289	-0.89 (13.9)	3.783	0.505
MDR297	-0.86 (14.8)	3.536	0.542
MDR298	-0.85 (15.0)	3.753	0.647
MDR307	-0.84 (15.3)	3.940	0.600
MDR303	-0.83 (15.5)	3.620	0.640
MDR292	-0.82 (15.6)	4.533	0.845
MDR301	-0.82 (15.7)	3.804	0.530
MDR296	-0.82 (15.7)	3.660	0.619
MDR293	-0.79 (16.5)	3.833	0.600
MDR305	-0.74 (18.0)	4.062	0.798
MDR299	-0.72 (18.6)	3.712	0.650
MDR304	-0.72 (18.8)	4.480	0.880
MDR295	-0.58 (23.3)	3.924	0.912
MDR049	-0.56 (24.3)	4.030	1.004
MDR291	-0.53 (25.2)	4.017	1.017
MDR308	-0.53 (25.2)	4.251	1.127
MDR290	-0.41 (30.2)	3.341	1.021
MDR294	-0.38 (31.4)	4.112	1.332

Table 7.9 Continued

Treatment	Logit percentage roots infected with take-all (back transformed means)	Number of roots/plant	Number of take-all infected roots/plant
Hexaploid wheat variety			
Hereward	-0.52 (25.5)	5.064	1.319
d.f.	85	85	
SED ²			
min.rep	0.177	0.288	0.215
max-min	0.153	0.249	0.186
F Probability	<.001	<.001	<.001
Grand mean	-0.732 (18.7)	4.075	0.799

¹ Data generated by R.J. Gutteridge before the PhD study commenced.

² 10 reps of control variety Hereward, five reps *T. monococcum* accessions.

In my PhD study a pot test was carried out on the sixteen *T. monococcum* accessions in the 2008-2011 field trials, as well as rye and triticale, and control pots of the hexaploid wheat variety Hereward. Despite mixing the collected soil with sand and increasing the dilution of sand/maizemeal inoculum to silver sand (1:50 dilution) take-all infection was still relatively low, with 33.2% roots infected for the control variety Hereward (Table 7.10). Despite the lower disease pressure there were significant differences between accessions. Rye and triticale were included to compare their known susceptibilities to take-all in the field as adult plants with performance at the seedling stage. Rye had the lowest level of infection and triticale was intermediate between rye and the hexaploid wheat variety Hereward, revealing that their partial resistance to take-all can be detected at the seedling stage. MDR217, MDR031 and MDR229 were the least infected with take-all (less than 20% roots infected in the pot test). In the field there was also a trend for these varieties to have lower levels of take-all infection. Partial resistance of MDR046 was more obvious in the field than in this pot test, although it was still one of the less infected accessions in the pot test. MDR026 was only included in the 2008 field trial, and was one of the most infected accessions, indicating that the pot test does not always accurately predict field performance. In contrast to the earlier pot tests carried out by Richard Gutteridge there were no significant differences in the number of roots infected with take-all per plant in the 2012 pot test (Table 7.10, $P = 0.172$), although there was a highly significant positive relationship between the number of take-all infected roots per plant and the percentage of roots infected ($R_s = 0.96$, $P < 0.001$, $n = 19$). The total number of roots per plant was also very weakly but significantly

correlated with the percentage roots infected with take-all ($R_s = -0.21$, $P = 0.04$, $n = 19$). In particular triticale has 4 or 5 more roots on average per plant than the other treatments but a similar number of take-all infected roots per plant, suggesting that the lower percentage roots infected could be partially due to the greater number of total roots per plant. Rye has one or two more roots per plant on average than the *T. monocooccum* accessions, although now the number of take-all infected roots per plant is also low.

Table 7.10. Susceptibility of *T. monocooccum* accessions to take-all infection in a seedling pot test in 2012.

Treatment	Logit percentage roots infected with take-all (back transformed means)	Number of roots/plant	Number of take-all infected roots/plant
<i>T.monocooccum</i> accessions			
MDR217	-1.82 (13.9)	6.413	1.480
MDR031	-1.62 (16.6)	7.113	1.233
MDR229	-1.42 (19.4)	7.920	1.620
MDR218	-1.38 (20.0)	6.500	1.460
MDR026	-1.27 (22.0)	7.980	1.860
MDR046	-1.12 (24.6)	7.860	2.080
MDR044	-1.00 (26.9)	6.324	1.824
MDR650 (PI355520)	-0.99 (27.1)	7.281	2.138
MDR025	-0.95 (27.9)	7.400	2.280
MDR002	-0.95 (27.9)	6.187	1.787
MDR286	-0.80 (31.0)	6.298	2.002
MDR037	-0.80 (31.1)	7.351	2.373
MDR043	-0.77 (31.6)	6.847	2.184
MDR308	-0.70 (33.2)	6.240	2.060
MDR232	-0.67 (33.9)	6.300	2.120
MDR280	-0.49 (38.1)	6.815	2.590
Rye	-3.54 (2.8)	8.256	0.302
Triticale	-2.05 (11.4)	11.289	2.071
Hereward	-0.70 (33.2)	6.943	2.480
d.f.	76	76	76
SED	0.585	0.297	0.633
F Probability	<.001	<.001	0.172
Grand mean	-1.21 (24.9)	7.227	1.890

7.3.3. *T. monococcum* DArT diversity analysis

Twenty *T. monococcum* accessions were analysed using diversity arrays technology by Triticarte, Australia (www.triticarte.com.au). The accessions were genotyped using over 1000 DArT markers. Polymorphism Information Content (PIC) values for the markers were generally quite low for the 20 accessions genotyped, with only 349 markers out of 1041 with a PIC value of between 0.4 and 0.5. The average PIC value was 0.30. Principal coordinate analysis and cluster analysis were carried out to look at grouping of accessions based on their genotype. This revealed clustering of the accessions as shown in Figures 7.1 and 7.2. The principal coordinate plot (Figure 7.1) shows the position of each accession in the space spanned by the first two coordinates of a relative Jaccard similarity matrix. The first two principal coordinates jointly explained 25.33% of the total data variance. The general susceptibility of the accessions to take-all is included in brackets in the principal coordinates plot. Figure 7.2 shows the hierarchal cluster analysis of genetic similarity between the accessions. Three separate samples of MDR037 were analysed using DNA prepared from different seed stocks. These are all shown to cluster very closely together (Figure 7.1), although there were still some differences between the seed stocks, indicating that the seed is not genetically identical. In the hierarchal cluster analysis their relative similarity is over 95% (Figure 7.2). The accession MDR298 was quite distantly genetically related to the other accessions. MDR298 has not been tested in the field for susceptibility to take-all. *T. monococcum* accessions MDR650 (PI355520) and MDR049 were also quite genetically dissimilar to other accessions and each formed their own group in the dendrogram tree (Figure 7.2). *T. monococcum* accessions MDR002 and MDR044 formed their own group, while the rest of the 16 *T. monococcum* accessions also formed one large grouping (Figure 7.2). The two accessions most resistant to take-all, MDR046 and MDR031, form their own subgroup with approx. 75% genetic similarity based on the 1041 DArT markers (Figures 7.1 and 7.2). Out of the most susceptible accessions in the *T. monococcum* field trials MDR043 and MDR308 are relatively genetically similar (>70% relative similarity), forming their own subgrouping, while MDR002 is not closely related. This provides evidence of some small agreement between genetic relationships based on the DArT markers and susceptibility to take-all. However, many of the other moderately resistant and susceptible accessions cluster together.

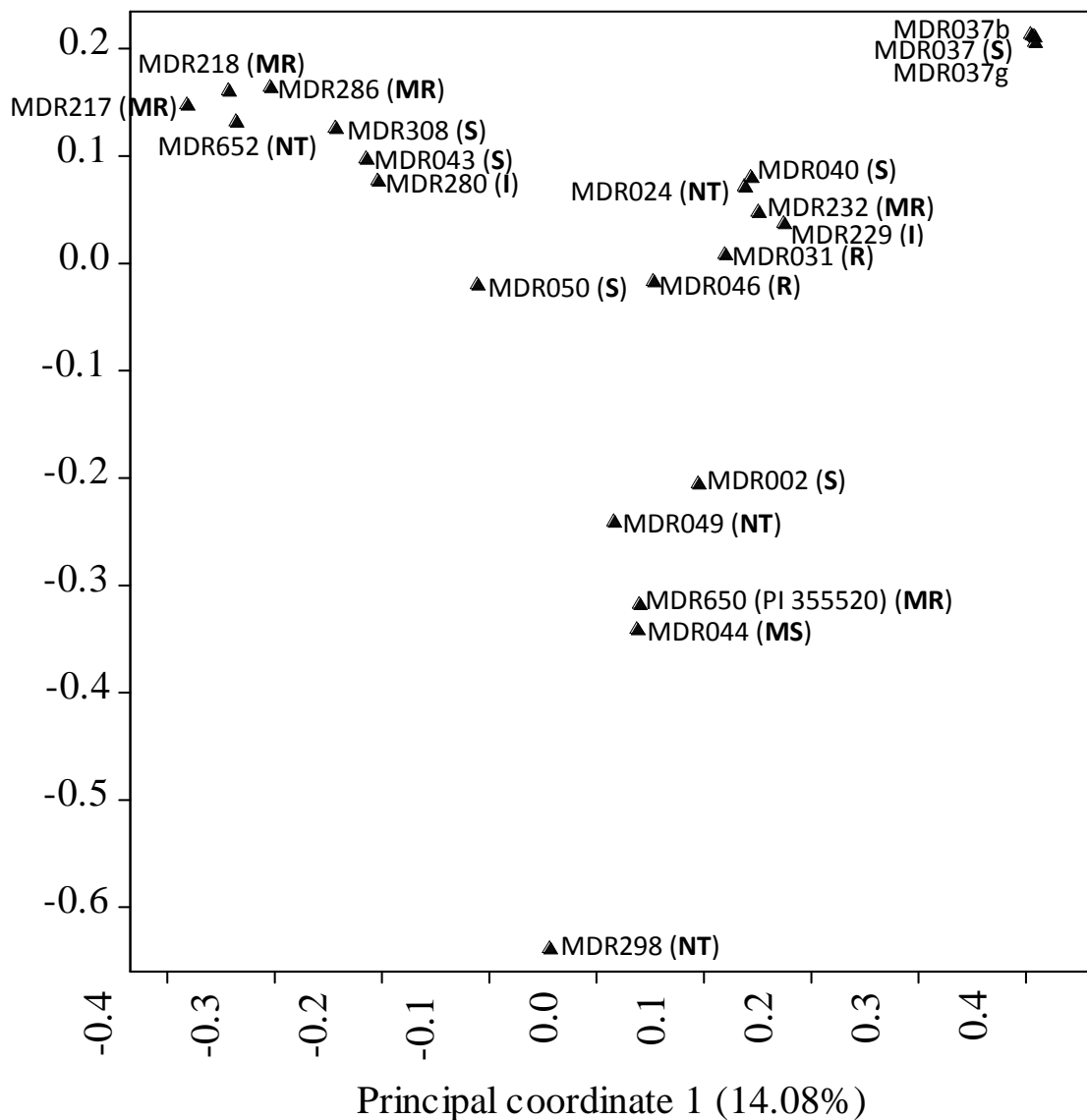


Figure 7.1. Principal coordinate analysis of 20 *T. monococcum* accessions based on 1041 DArT markers. The accession codes and susceptibility to take-all are inserted in the figure. Susceptibility to take-all is based on the field screening reported in this chapter. Accessions were classified as susceptible (S), moderately susceptible (MS), moderately resistant (MR), resistant (R), inconsistent performance in different field trials (I), and not tested in the field (NT).

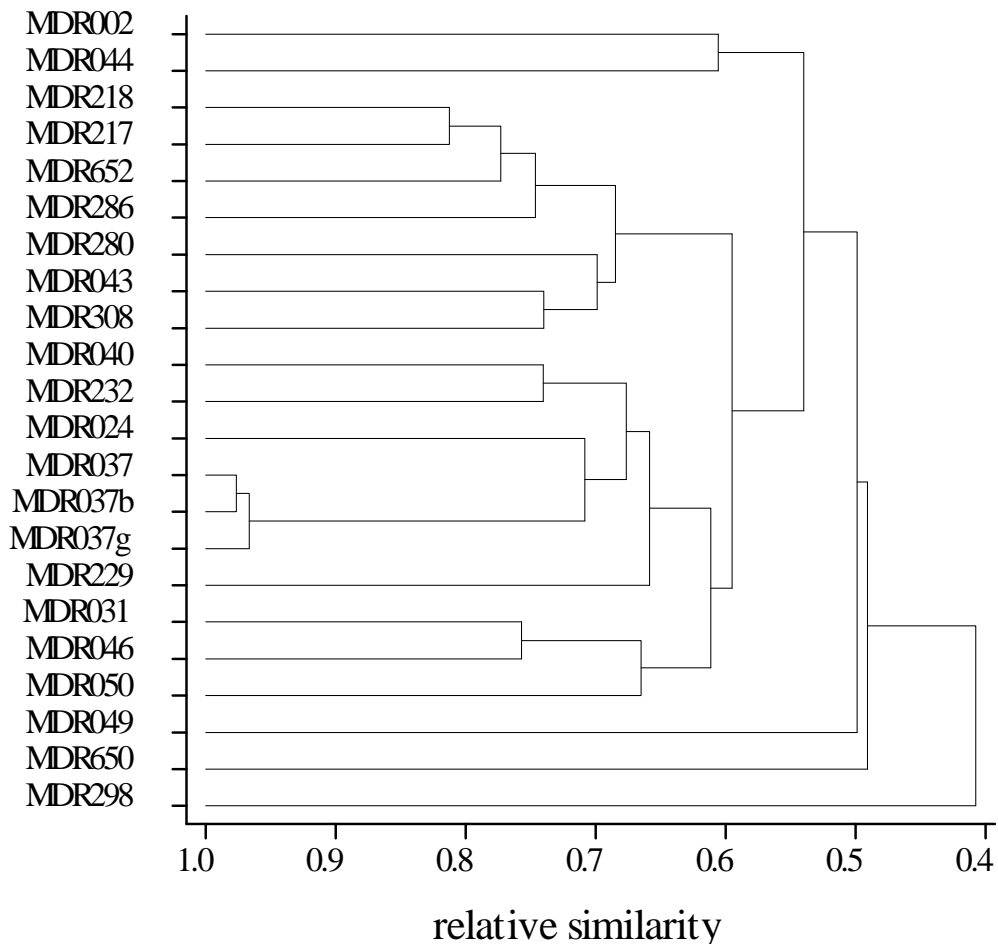


Figure 7.2. Dendrogram of genetic similarity among the 20 *T. monocoicum* accessions constructed based on group average cluster analysis.

7.3.4. Response of *B. distachyon* ecotypes to take-all

All of the five *B. distachyon* ecotypes were highly susceptible to the take-all fungus and severely infected (Table 7.11; Figure 7.3). *Brachypodium* plants were also more susceptible than the wheat plants in the *Ggt*-wheat pathogenicity test (see Chapter 5: Characterisation of a new *Ggt* isolate collection; Mean % roots infected on wheat plants grown from untreated seed with silthiofam sensitive isolates and silthiofam resistant isolates was 71.5% and 73.9% respectively). None of the five *B. distachyon* ecotypes exhibited a resistant response to *Ggt* root infection, whereas ecotypes ABR 5 and ABR 6 are known to exhibit resistance to leaf infection with the rice blast fungus *M. oryzae* (Routledge et al., 2004).

Table 7.11. Pathogenicity of silthiofam sensitive (S) and resistant (R) *Ggt* isolates on five *Brachypodium distachyon* ecotypes.

Accession	Ecotype	Mean % roots infected ¹		
		S	R	Control ²
MDR672	ABR 1	96.0	98.0	0.0
MDR673	ABR 2	100.0	97.5	0.0
MDR674	ABR 3	100.0	98.3	0.0
MDR675	ABR 5	94.6	97.8	0.0
MDR676	ABR 6	93.8	100.0	0.0

¹ Mean of five replicates (5 different sensitive isolates each treated as a separate replicate, 5 different resistant isolates each treated as a separate replicate).

² Control, non colonised agar.



Figure 7.3. Pathogenicity test *Brachypodium distachyon* seedlings infected with take-all; left = control plants, right = severely infected seedling with blackened roots, yellowing leaves and stunted growth.

7.4. Discussion

Five years of field trials were carried out to evaluate the resistance to take-all of thirty-four *Triticum monococcum* accessions under UK conditions. The first two trials in 2006 and 2008 were carried out before the start of the PhD study. The field trials in 2006, 2008 and 2009 were highly conducive to disease development. In contrast the 2010 and 2011 trials were lower disease pressure years. In these experiments accessions were compared with the performance of the fully susceptible hexaploid wheat variety Hereward and the differing known susceptibility of the species controls oats, rye and triticale. An initial screening of 27 *T. monococcum* accessions in the field trial in 2006 discovered a range of susceptibilities to take-all in this diploid wheat species. These initial results revealed that some *T. monococcum* accessions had a significantly lower amount of take-all disease than the hexaploid wheat Hereward and so could contain potentially useful sources of resistance to the take-all fungus. This was in contrast to a previous study by Mielke (1974) who reported that *T. monococcum* as a species was severely infected under field conditions. In 2006 and 2007 my supervisor Richard Gutteridge also completed 3 pot tests to explore the susceptibility of *T. monococcum* at the seedling stage, and to select accessions for further screening in the field trials. At the seedling stage a range of susceptibilities to take-all were also discovered. From the results of these pot tests the *T. monococcum* accessions MDR025, MDR026, MDR031, MDR046, MDR229 and MDR232 were revealed as showing potential partial resistance to take-all and so selected by Richard Gutteridge for inclusion in the 2008 field trial. In this trial accessions MDR031 and MDR046 displayed the lowest level of take-all disease. *T. monococcum* accessions MDR229 and MDR232 also displayed some partial resistance to take-all. The other two of the promising accessions in pot tests (MDR025 and MDR026) both had relatively high take-all indexes in the field. In the pot test a mixture of 10 or more *Ggt* isolates is used to inoculate seedlings as a representation of field populations of take-all. In the field there are undoubtedly many more *Ggt* isolates and it is possible that there are different interactions of isolate genotype and host genotype, so that MDR025 and MDR026 were no longer able to restrict infection under the greater variation of isolates in the field.

Previously relatively large significant differences between wheat varieties have been reported from individual field experiments, but these have not been reproducible across sites and seasons. For example in the three elite winter wheat and susceptibility to take-

all field trials (Chapter 6), there was generally not a consistent ranking of varieties between the trials. Differences may be masked by the interaction of environmental conditions with host and/or pathogen, disease ‘patchiness’, inaccurate assessment, possible interaction between pathogen genotype and host genotype, and possible interactions between previous host genotype in the rotation and the genotypes tested. The challenge has been to find resistance that is consistent across different sites and seasons. After finding promising accessions in Richard Gutteridge’s pot test and the 2006 and 2008 field trials it was then important to test material over multiple years to identify accessions with consistent expression of take-all resistance. Field trials were therefore continued over a further 3 years from 2009-2011 during my PhD study. Across these trials in both high and low disease pressure years MDR046 and MDR031 were consistently the very best material tested. These two accessions had a level of take-all resistance intermediate between the species controls rye and triticale. No accession has been found to contain the very high resistance/immunity to *Gaeumannomyces graminis* var. *tritici* shown by the control species oats. Other accessions were consistently highly susceptible to take-all. These data are summarised in Table 7.12. This information will be used to select contrasting parent accessions for crossing to create mapping populations and so investigate the genetic basis of this trait. Two varieties, MDR229 and MDR280, had quite unreliable phenotypes in different years. In the high disease years (2006 and 2008) they were moderately resistant while in the low disease pressure years (2010 and 2011) they were more susceptible.

Table 7.12. Summary of susceptibility of *T. monococcum* accessions to take-all in field trials at Rothamsted 2006 & 2008-2011. Accessions included in only one year of trials were excluded as it is not possible to determine how consistently they perform under field conditions.

<i>T. monococcum</i> accession	No. of years in trials	Susceptibility to take-all ¹
MDR002	5	S
MDR031	3	R
MDR037	5	S
MDR043	3	S
MDR044	2	MS
MDR046	4	R
MDR217	4	MR
MDR218	4	MR
MDR229	4	I
MDR232	3	MR
MDR280	4	I
MDR286	4	MR
MDR308	5	S
MDR650 (PI355520)	3	MR

¹ Accessions were classified as susceptible (S), moderately susceptible (MS), moderately resistant (MR), resistant (R) or with inconsistent performance in different field trials (I).

Upon examining the data from pot and field tests, a number of other potentially partially resistant accessions were apparent that have not been adequately tested in follow up field trials. This may have been due to limited seed availability. In particular MDR279 was one of the best accessions in the 2006 field trial but since 2006 has not been included in further trials. In pot test 3 in 2007 there were also two accessions (MDR302 and MDR306) that stood out with low levels of take-all infection which were not included in further field screening. These accessions are therefore priorities for future pot and field screening work. It would be useful to identify a number of different resistance sources with the possibility of discovering different mechanisms of resistance. If the genetic regions controlling resistance could be identified this could allow the pyramiding of resistance sources to achieve a level of resistance that could be more useful for deployment.

In 2012 I carried out a pot test on the 16 *T. monococcum* accessions included in the 2008-2011 field trials. Rye and triticale were also included and the known susceptibility of rye and triticale in the field was found to be expressed at the seedling stage in the pot

test. There was a general agreement of the ranking of varieties in the pot test and field trials, but the differences in susceptibility were not as apparent at the seedling stage in the pot test. Also, the susceptibility of some of the accessions was not reliably expressed in the pot test, indicating that take-all susceptibility at the seedling stage is not a reliable indicator of adult plant susceptibility in the field. In order to accurately identify resistance sources field phenotyping over multiple years is therefore the required procedure. However, the pot test has been a useful way to select accessions for field phenotyping from a large collection of stock accessions.

The DArT marker analysis shows that *T. monococcum* accessions are genetically differentiated. Principal coordinate analysis of 20 *T. monococcum* accessions revealed that their susceptibility to take-all is not generally that closely associated with whole genome diversity. Interestingly the two most resistant accessions, MDR046 and MDR031, did form their own sub-cluster, perhaps suggesting a common source of genetic resistance in these accessions. The pedigrees of accessions are unknown but the country of origin for MDR046 is Romania and MDR031 is Turkey, showing that these two accessions were not collected from the same geographical region. Other moderately resistant accessions were not very similar genetically, indicating that a range of genetic sources of resistance are likely to be found within *T. monococcum*.

A number of hexaploid and tetraploid wheat varieties were included in the field experiments. Most of the hexaploid varieties were only included in one or two years of experiments so it was not possible to determine reliably their phenotypes. Overall, the data demonstrate the generally high susceptibility of modern wheat varieties to take-all. However, there were sometimes large significant differences between the hexaploids in individual years. The varieties Hereward and Robigus, tested in all five years of the diploid wheat trials, were relatively consistent as the two of the most susceptible hexaploids. The variety Solstice, tested in the 2009-2011 trials, was less susceptible. In these trials Solstice had a take-all index similar to the triticale control. However, Solstice did not stand out as a good modern variety in terms of take-all susceptibility in the elite winter wheat third wheat variety trials described in Chapter 6, indicating that its phenotype is not reliably expressed between different sites. Also, the least susceptible *T. monococcum* accessions were still lower than Solstice in the 2009-2011 diploid wheat field trials, suggesting that *T. monococcum* could be a useful source of resistance to take-all in the genetic improvement of hexaploid wheat. In the low disease pressure

years, 2010 and 2011, the hexaploid varieties as a whole perform relatively well with quite low take-all indexes. In contrast the five tetraploid wheat varieties appear particularly susceptible to take-all at both high and low disease pressure, in 2009 and 2010 respectively. The species *Aegilops speltoides*, a progenitor of tetraploid wheat, was only tested in 2010 but in this year was more susceptible than all of the hexaploid wheats, but less susceptible than the tetraploid wheats. This provides evidence that the tetraploid wheat lineage is not likely to be a useful source of resistance to the take-all fungus.

Many studies have demonstrated that rye is highly resistant to take-all, compared with wheat (Nilsson, 1969, Scott, 1981). However genetic exchange between rye and wheat is relatively difficult and the resistance of triticale (the wheat x rye cross) is usually closer to wheat than rye (Scott, 1989). There has also not been consistent variation between rye cultivars making the genetic analysis of resistance to take-all in rye not possible. In comparison *T. monococcum* is more closely related to hexaploid wheat (*T. aestivum*) and useful traits can be introgressed from *T. monococcum* into hexaploid wheat (Valkoun, 2001). Genetic loci conferring resistance to leaf rust and powdery mildew have already been successfully introgressed into tetraploid and hexaploid wheat (Shi et al., 1998, Vasu et al., 2001, Xu et al., 2008). Variation between accessions in their susceptibility to take-all and its smaller diploid genome also make *T. monococcum* ideal for genetic studies of resistance. Such genetic analysis should reveal whether the trait is control by a single locus or multiple loci. Jing et al. (2008) found that resistance to the foliar pathogen *Mycosphaerella graminicola* in *T. monococcum* was caused by a single genetic locus. This makes it easier to transfer into hexaploid wheat. If multiple genetic loci control the trait then multiple introgressions would need to be carried out.

The mechanism(s) of partial resistance to take-all within *T. monococcum* are also not known. Interestingly in the literature it has been suggested that natural root cortex cell death could influence species or cultivar susceptibility to take-all. In laboratory experiments Liljeroth (1995) found that the rate of natural root cortical cell death was slower in *T. monococcum* than hexaploid wheat. Root cortex death was also found to be much faster in wheat than barley, rye or oats; and triticale was found to have a root cortex death rate intermediate between rye and wheat (Liljeroth, 1995). This ranking of species in terms of root cell death is the same as their ranking in terms of susceptibility to take-all. However, there is as yet no clear evidence that *Ggt* benefits from root cell

death. Kirk (1984) hypothesised that *Ggt* could benefit from natural root cortex death by increasing its food supply before infecting the living cell tissues underneath. Under field conditions Deacon and Henry (1981) found up to five dead root cortex cell layers in the top 5.4 cm of the seminal root axes of wheat by the middle of April (out of a maximum of 6 root cortex cell layers). Deacon and Lewis (1982) have suggested that natural root cortex death is an important influence on susceptibility of wheat varieties to another root pathogen, *Cochliobolus sativus* (Common root rot). They found that wheat varieties that were most resistant to common root rot had slower rates of root cortex death than susceptible varieties. Only one accession of *T. monococcum* was used in the study by Liljeroth (1995) so it is unknown whether there is variation in this trait among *T. monococcum* accessions. However, hexaploid wheat varieties have been shown to have small differences in their rates of root cortex death (Henry & Deacon, 1981). Studies of rates of root cell death in different *T. monococcum* accessions would allow comparison with their susceptibilities to take-all.

One of the main aims of this work going forward is to further characterise the resistant and susceptible *T. monococcum* accessions identified in field screening as a first step towards understanding the underpinning resistance mechanisms. Low disease scores in the field may be due to resistance mechanisms that hinder root colonisation or spread of disease. Low disease scores may also be the result of disease escape/dilution by disease induced root production or inherent rooting ability. Characterisation should be carried out to permit the separation of tissue-based resistance mechanisms versus rooting ability. This can be carried out by counting the number of roots of *T. monococcum* accessions in the presence and absence of disease and assessing correlation of these data with disease scores for the same samples. Rooting ability at different times of the season could also have an important influence on disease. As mentioned in Chapter 6 high root production early in the season could increase primary infections as it is more likely for roots to come into contact with inoculum in the soil. The amount of primary infection could then go on to influence the rate of secondary infections, increasing the rate of disease development when there are many original infected roots. Rooting ability can therefore have a complex effect on disease severity and the amount of damage caused. Epidemiology studies as in Chapter 6 would be a useful way to investigate disease development during the season. So far in the *T. monococcum* field trials only the final disease severity towards the end of the season has been evaluated.

Characterisation of tissue-based resistance to take-all could be carried out using microscopy studies. Roots of plants from disease tests could be examined for the presence of runner hyphae and appropriate staining and light or UV microscopy used to identify plant defence responses and the progress of infection through cell layers. In the summer of 2010 I jointly supervised with Allison van de Meene (Head of Bioimaging at Rothamsted) a 10 week BBSRC bursary student Joseph Whittaker. The aim of the bursary project was to investigate the infection biology of *Ggt* in the resistant and susceptible *T. monococcum* accessions, MDR031 and MDR037. A novel hydroponic take-all infection system, developed by a colleague at Rothamsted, Bob Pritchard, was used in the bursary project. The hydroponic method proved to be a quick way of infecting plants and was useful for bioimaging purposes. However, the resistant diploid wheat accession did not show a difference in the level of infection to the susceptible accession when infected in this manner. The hydroponic system used does not therefore seem to be a good screen for resistance, perhaps because of over saturation of the roots with take-all fungus. Or alternatively, because the resistance responses are only effective in more mature plants. Joseph used various light microscopy and histochemical techniques to evaluate both the fungal infection pathway and the host response in the two *T. monococcum* accessions. This included staining root sections with chlorazole black E and trypan blue to reveal fungal hyphae within the root (Resendes et al., 2001, Sesma & Osbourn, 2004). Various other stains were used to detect host defence response. These included phloroglucinol (Speakman & Lewis, 1978, Penrose, 1987b) and safranin (Davis, 1925) to detect lignin, calcofluor to detect cellulose and callose, and aniline blue to detect callose (Mylona et al., 2008). Skou (1981) described the formation of cell wall structures around and in front of advancing *Ggt* hyphae to slow down the spread of the fungus. These structures have been called lignitubers. Kang et al. (2000) found that lignitubers contained callose, cellulose, xylan and lignin. Previously Speakman and Lewis (1978) and Penrose (1987b) have both stained wheat root sections with phloroglucinol to investigate the role of lignification of cell walls in wheat roots invaded by *Ggt*. Speakman and Lewis (1978) reported that lignification of cells walls was not greatly increased by *Ggt*. However, Penrose (1987) found that cell wall thickening was a common response in seminal roots infected with *Ggt* and was associated with lignin deposits. Wheat genotypes were also shown to differ in their ability to lignify and it was suggested that this could restrict pathogen invasion. During his BBSRC bursary project Joseph did not find any obvious evidence of

different host defence responses in MDR031 and MDR037 infected in the hydroponic system. Further work could involve modifications to the system to test whether consistent discrimination between wheat genotypes can be achieved using the hydroponic method.

In this study the developing model grass species *Brachypodium distachyon* was also evaluated for its susceptibility to take-all. *B. distachyon* appears to be fully susceptible to take-all; no *Ggt* isolate induced a resistant response from any *B. distachyon* ecotype. Screening a wider range of *B. distachyon* ecotypes could be useful to find different response phenotypes to *Ggt* for future investigation. From this initial small study the interaction of *B. distachyon* with *Ggt* does not appear at present to offer the opportunity for investigating resistance mechanisms to *Ggt*.

In summary this study has demonstrated consistent contrasting susceptibilities to take-all over multiple field trial years within the diploid wheat species *Triticum monococcum*. Future studies will focus on defining the genetic basis of this trait and the introgression of resistance into modern hexaploid wheat.

CHAPTER 8: GENERAL DISCUSSION

8.1. Project summary

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 and seedling pot tests.

A first wheat field trial in 2009 demonstrated a range of inoculum building abilities within current National List winter wheat varieties. No variety was significantly better than the previously identified low TAB variety Cadenza, although low levels of inoculum build-up were found in other varieties. The underlying mechanisms influencing the TAB trait are unknown. The field data (Chapter 3) in combination with the pedigree and molecular marker analysis (Chapter 4) suggests that there are multiple genetic sources of the low TAB trait within current elite wheat varieties. This could be useful from a plant breeding perspective as it could allow different sources to be combined into new varieties. Rotation trials, set up to investigate the significance of this finding, revealed that sowing a low TAB first wheat variety resulted in generally lower take-all and higher yields in the following second wheat crop. 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 (discussed further in section 8.3). Then the time consuming phenotyping would occur only in the later generations when fewer lines remain.

In common with previous literature, a range of susceptibilities of hexaploid wheat varieties to take-all were found in third wheat field trials but differences were not very consistent between sites and years (Chapter 6). This demonstrated that the inoculum build-up trait was not related to the susceptibility of wheat varieties to take-all root infection in third wheat field trials. There was some evidence of partial resistance to take-all in a limited number of current NL wheat varieties but it is not yet clear whether this would result in improved yields in a take-all risk situation. In contrast to the overall

relatively small and inconsistent differences of hexaploid wheat varieties to take-all (Chapter 6), a more consistent range of susceptibilities to take-all were demonstrated for the diploid wheat *Triticum monococcum* under field conditions (Chapter 7). High levels of resistance were found in some accessions that could be useful in improving the resistance of hexaploid wheat. So far the genetic basis and mechanisms of resistance in some of the *T. monococcum* accessions are not known. Whole genome genotyping suggested that two of the most resistant accessions were similar genetically, but other moderately resistant accessions were not similar. The seedling pot test method was explored as a screen for resistance to take-all by comparing the performance of varieties in the pot test with their resistance in the field. In general, differences between varieties were less pronounced in the seedling pot tests and field performance could not be reliably predicted based on this screen. Field phenotyping is therefore necessary for both traits, take-all inoculum build-up and resistance. Multiple years of trials are also essential to assess both traits as some varieties perform quite inconsistently, perhaps due to environmental interactions.

8.2. Further work

Based on the results of this PhD project, further work is planned to investigate and characterise both the take-all inoculum build-up trait and the susceptibility of hexaploid wheat varieties and *T. monococcum* to take-all.

As discussed in Chapter 3 the inoculum building ability of twelve of the forty-five elite NL wheat varieties is being tested in further field trials to investigate how consistently varieties perform. This is because only one year (2009) out of three field trials generated useful information on this trait within the PhD project. Epidemiology studies are being continued to identify the critical time periods during which differences between varieties occur and above ground traits are also being recorded to link with the TAB trait. These trials are now being over-sown in the second year with a single wheat variety to monitor the effect of the TAB phenotype in year 1 on take-all and yields in a second wheat crop. Further work is also planned to investigate the origins of the TAB trait in the Cadenza and Avalon pedigrees. Based on the pedigree analysis in the PhD project seed of 80 varieties was obtained for field and molecular analysis. Seed is being bulked up in the field ready for trialling from autumn 2013.

Based on results within the WGIN project and this PhD project, a five year (2010-2015) new joint project with three of the UK based plant breeding companies was funded by BBSRC and the Technology Strategy Board, called 'Protecting second wheats through the reduction of low TAB'. In this new project work is being carried out to identify new breeding lines showing the low TAB phenotype, which could be selected for commercialisation by the respective breeding company. Work is also on-going within the WGIN project and BBSRC-TSB project to confirm and fine-tune the location of the QTLs controlling the trait in Cadenza. This new project will use the extended A x C mapping population, developed at Rothamsted, which consists of 582 new A x C lines (WGIN newsletter May 2010; <http://www.wgin.org.uk/stakeholders/newsletters.php>). The A x C lines in the extended mapping population have already been screened, by the breeders, with a high density of molecular markers across the QTL regions to identify recombinants within these regions. In the public domain, 1054 markers are available for the A x C population (Allen et al., 2011). Specific A x C recombinant lines will then be selected for field phenotyping and analysis to try to reduce the size of the QTL interval and to identify more tightly linked markers. The main aim would be to identify diagnostic markers that could be used after the project by the plant breeders in their programmes to screen new material for this trait as well as advance the trait within the later stages of existing breeding programmes.

As described briefly in Chapter 7 mapping populations are being developed to investigate the genetic basis of resistance to take-all in some of the *T. monococcum* accessions. Further work could involve using light, UV-autofluorescence and scanning electron microscopy to assess where take-all infections become arrested in the most resistant accessions and so begin to characterise susceptible and resistant accessions. This would build on the work started in Joe Whittakers' BBSRC summer project described in Chapter 7. Further exploration of the conditions of the hydroponic system and soil pot test to distinguish between susceptible and resistant phenotypes is required. So far assessment of take-all is carried out by visual examination. Further work could involve the development of a qPCR method to explore fungal biomass levels.

Work is also planned to explore whether the related rice blast fungus *Magnaporthe oryzae*, which is known to infect wheat roots under experimental conditions (Dufresne & Osbourn, 2001), can be used as a surrogate for *Ggt* in hydroponic and pot tests. *Magnaporthe oryzae* and *Ggt* infection will be compared in susceptible and resistant *T.*

monococcum accessions. *Magnaporthe oryzae* is readily transformable, unlike *Ggt*, and a range of reporter strains expressing useful reporter genes, such as the green fluorescent protein (GFP) are already available. The use of these strains could greatly assist the exploration of the *in planta* infection process in wheat. In the third year of my PhD I prepared applications that were submitted to the Health and Safety Executive (HSE) and the Plant Health and Seeds Inspectorate (PHSL) to use a GFP tagged strain of *M. oryzae*. Receipt of notification of this application was received from HSE, but a follow up question from HSE, received in January 2011, regarding the possibility of symptomless *M. oryzae* colonisation from wheat roots to the leaves, followed by symptomless sporulation on the wheat leaves, has still to be successfully answered before any experiments can take place in the future.

8.3. Using QTL mapping information to develop new wheat varieties

The aim of plant breeding is to improve the quality and performance of agricultural crops by combining traits such as high yield, with resistance to biotic and abiotic stresses and producing varieties that are adapted to the local environment and end use. There is always a need for new varieties due to changes in disease pressures and growing conditions, changing markets and due to the impact of new agricultural and environmental policies (British Society of Plant Breeders, BSPB, www.bspb.co.uk). Wheat breeding for genetic resistance to disease is considered one of the best control strategies for a range of diseases, including the fungal Fusarium and Rust diseases, and viruses such as barley yellow dwarf virus (Nelson, 1973, Miedaner, 1997, Stuthman et al., 2007, Kosova et al., 2008, Park, 2008). Resistance to many plant diseases is genetically complex and quantitative (Young, 1996). Quantitative disease traits show continuous (as opposed to categorical) phenotypic variation in genetically segregating host populations (St Clair, 2010). The take-all inoculum build-up trait is an example of a quantitative trait, displaying continuous variation segregating in the Avalon x Cadenza mapping population. The partial disease resistance of *T. monococcum* to take-all is also likely to be quantitative. Quantitative traits are often controlled by many different genes and the regions of the genome which contribute to the trait are called quantitative trait loci (QTL). Each given QTL can contribute to disease resistance in different amounts and this is expressed as the percentage of phenotypic variation accounted for. If a QTL accounts for over 20% of the phenotypic variation it is usually classified as a major QTL (St Clair, 2010). Molecular markers differentiate individuals based on

polymorphisms in the genome (Lefebvre & Chevre, 1995) and QTLs are identified based on their linkage to molecular markers. There are various molecular markers that have been developed to identify genetic polymorphisms between individuals and for use in QTL mapping. The most common markers are single nucleotide polymorphisms (SNP) and simple sequence repeats (SSR) (Mackay et al., 2009). The development of abundant polymorphic markers such as SNPs and SSRs has facilitated the development of high density marker linkage maps and allowed the genotyping of many individuals in segregating populations used to study quantitative traits. Segregating populations are developed from a cross between two individuals that differ in the trait of interest. For example in the Avalon x Cadenza mapping population, the Avalon parent is a consistently high building variety and Cadenza a low building variety, so the population of crosses show segregation for the TAB trait. QTL mapping is carried out by genotyping individuals in a variable population with molecular markers that cover the whole genome and also phenotyping each individual for the quantitative trait of interest. The genetic and phenotype datasets are then statistically analysed using QTL mapping software to identify significant associations between the molecular polymorphisms and phenotype (St Clair, 2010).

The genetic information revealed in QTL mapping studies can have practical applications in wheat breeding programmes to produce new varieties by marker assisted selection (MAS). This involves using markers that are tightly linked to the trait of interest to track the trait and select individuals in plant breeding programmes. MAS allows the elimination of unwanted plant genotypes at the seedling stage to speed up the plant breeding process by reducing the total number of lines to be phenotyped, and can be a way of selecting parent varieties for breeding programmes. Xu and Crouch (2008) state that the development of MAS is useful for traits that are difficult to select in traditional phenotypic selection breeding programmes. This could be because they are expensive, labour intensive and/or time consuming to measure. MAS would be very useful to breed varieties with the low TAB trait as take-all build-up can only be phenotyped in the field, it's expression is vulnerable to soil and environmental conditions and it is labour and time intensive to phenotype individuals. Before information in QTL mapping studies can be successfully used for MAS it is generally necessary to confirm, validate and fine map the QTL to identify markers that are tightly linked enough to the trait to act as diagnostic markers in selection programmes (Collard & Mackill, 2008). An example of the successful use of MAS in a wheat breeding

programme is the selection of the *Fhb1* major QTL for resistance to Fusarium head blight (FHB) (Anderson et al., 2007, Pumphrey et al., 2007). The *Fhb1* QTL, originating in the variety Sumai 3 on chromosome 3B, was reported in two separate mapping populations by Anderson et al. (2001). Other researchers also confirmed the major effect of this QTL (Yang et al., 2003). Pumphrey et al. (2007) validated the QTL in different genetic backgrounds by developing Near-Isogenic wheat Lines (NILs) from 13 breeding populations using SSR markers that were tightly linked to the QTL. Testing of these lines in four replicated field trials and a greenhouse screen revealed that the Sumai 3 allele at *Fhb1* had a consistently large effect on resistance and that SRR markers could be successfully used to select for increased resistance to FHB.

Marker assisted selection is useful to pyramid multiple QTL/genes for a single disease resistance trait, helping to create more durable disease control than single gene resistance. For example Sreewongchai et al. (2010) used MAS to combine four rice blast resistance QTLs into a single rice genotype. In barley Castro et al. (2003) incorporated two QTLs for quantitative resistance and a single gene for qualitative resistance against stripe rust into the same barley genotype.

Markers that are linked to a QTL can also be used in marker assisted backcrossing (MAB) in plant breeding programmes. This is a process used in plant breeding to incorporate genes of interest into an elite variety (Collard & Mackill, 2008). Using tightly linked markers can help reduce the size of the introgressed chromosome segment. The imprecise insertion of large QTL regions can cause problems due to 'linkage drag' where deleterious genes are also transferred. MAB is also used to select for individual backcrosses that are genetically most like the recurrent parent, except at the target QTL positions where the donor genome is selected. Markers across the whole genome that are unlinked to the QTL of interest are used to select for the recurrent parent. This process is called background selection. Kuraparthi et al. (2011) introgressed the leaf rust resistance gene Lr58 from *Aegilops triuncialis* into two winter wheat cultivars in America using MAB. If the genetic basis of resistance of *T. monococcum* to take-all can be elucidated, MAB would greatly speed up and help the process of transferring this resistance into hexaploid wheat. Background selection is particularly important when incorporating traits from other species and wild relatives like *T. monococcum* which are very different from the recurrent parent. It should be noted that advanced breeding lines developed from MAS and/or MAB will still require

testing in field experiments over several seasons to test for a consistent improvement of field performance before any new varieties are released. Therefore release of new varieties after MAS will still take several years.

In the literature no QTLs for resistance to take-all have been reported. Genetic analysis of the low TAB trait in modern hexaploid wheat and take-all resistance in *T. monococcum* is the first step towards the creation of new varieties containing these traits.

8.4. Sequencing the wheat (*Triticum aestivum*) genome

Paux et al. (2008) state that ‘genome sequencing is the foundation for understanding the molecular basis of phenotypic variation’. The first sequenced genomes were the model species *Arabidopsis thaliana* and the major cereal crop species rice, *Oryza sativa*. The International Rice Genome Sequencing Project (IRGSP) was set up in 1998, with a draft sequence available in 2002 and the sequence finished in 2005 (Matsumoto et al., 2005). Since then the cereal species maize (*Zea mays*) and sorghum (*Sorghum bicolor*) have been sequenced and projects are underway to sequence the barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) genomes (Feuillet et al., 2011). Crop genome sequencing projects and the development of high-throughput genotyping platforms should help to provide substantial amounts of new data for use in marker development, QTL mapping projects and map-based cloning of QTL and genes of interest, speeding up the process of breeding for crop improvement. The International Wheat Genome Sequencing Consortium (IWGSC) (www.wheatgenome.org) was set up in 2005 to coordinate the sequencing of the hexaploid bread wheat genome. So far wheat chromosome physical maps have been constructed for nine chromosomes (1A, 3A, 1B, 3B, 6B, 1D, 3D, 4D, 6D) and a reference sequence for the largest wheat chromosome, chromosome 3B, is available. Physical maps are useful to establish links between the underlying sequence and previously identified QTL and genes of interest (Philippe et al., 2012). The partially sequenced wheat genome and an abundance of expressed sequence tagged (EST) information also provides a way of investigating gene function. Using sequence data, genes can be isolated and / or reconstructed using various bioinformatic methods and their function studied by over-expression, under-expression and deletion methods. This could be achieved by the generation and testing of stable wheat transgenics (Jones & Shewry, 2008) or via the use of new transient technologies,

for example virus induced gene silencing (Lu et al., 2003, Scofield et al., 2005, Lee et al., 2012).

Next generation sequencing (NGS) technologies such as Roche/454 (www.454.com) and Solexa/Illumina (www.illumina.com), which allow DNA sequence data to be generated at a much faster rate and lower cost than the traditional Sanger sequencing technology, are also predicted to assist greatly crop genetics and plant breeding (Varshney et al., 2009). NGS technologies can be applied to resequencing projects once the reference wheat sequence is finished. This could allow the sequencing of wheat genotypes of interest to the wheat breeding companies, for example the parent varieties of mapping populations (Varshney et al., 2009). It is possible to realign new sequence data to the reference genome so that genetic variants between the genotypes can be recognised allowing the discovery of polymorphic genome wide SNP molecular markers for these genotypes. For example Lai et al. (2010) resequenced six elite maize inbred lines, discovering over 1,000,000 SNPs for use in future molecular breeding. Comparison to an annotated reference genome also allows researchers to predict whether an SNP is located within a gene of interest and so whether the SNP is causing a particular phenotype. Or researchers can build-up haplotypes of the distribution of polymorphisms around loci of interest. NGS technology could also be used to generate sequence data and molecular markers for traits of interest in wild crop relatives, to improve introgression into modern wheat varieties.

8.5. Sequencing the *Ggt* genome

In October 2010, the draft genome assembly of *Ggt* strain R3-111a-1 of US origin was made available by the Broad Institute, USA (www.broadinstitute.org). In April 2011 the annotated release of the *Ggt* genome was then published with 14,463 predicted genes spread over the 43.62 Mb genome. The *Magnaporthe poae* (strain ATCC 64411) genome is also being sequenced and annotated as part of the same project to build-up a comparative *Magnaporthe* database. This database also includes the partially assembled sequence of *Magnaporthe oryzae* (strain 70-15) which was originally published in 2005 (Dean et al., 2005). All three of these fungi are economically important plant pathogens in the family Magnaporthaceae. Both *Ggt* and *M. poae* are soil-borne fungi infecting plant roots while *M. oryzae* is primarily an air-borne fungal pathogen but has also been shown to be able to infect roots (Besi et al., 2008). *M. poae* causes patch disease of grasses in the genera *Poa*, *Festuca* and *Agrostis*, commonly used as turf

grasses on golf courses and parks. In a similar way to *Gaeumannomyces graminis*, infection of turf grasses with *M. poae* is characterised by ectotrophic brown runner hyphae and the formation of hyphopodia infection structures to penetrate root tissue (Tredway, 2006). *M. oryzae* causes the globally important disease rice blast. The *M. oryzae*-rice interaction has become a model system for studying plant-fungal interactions with a large number of genomic resources developed for both the pathogen and host (Besi et al., 2008). The genome sizes and predicted number of genes of the sequenced strains of *M. oryzae* and *M. poae*, 41.03 Mb with 12,827 genes and 39.5 Mb with 12,169 genes respectively, are similar to that of the sequenced *Ggt* strain. Once the genome sequences of *Ggt* and *M. poae* are finished comparative genomic analyses between these three species should reveal insights into pathogenicity of these fungi on different hosts and tissues. Comparative genomics can also be used to compare the genomes with other sequenced plant pathogens. As of August 2012 according to the Comprehensive Phytopathogen Genomics Resource (CPGR) database there are 138 annotated plant pathogen genomes (<http://cpgr.plantbiology.msu.edu/index.html>). This includes other important fungal plant pathogens of wheat in the UK such as *Mycosphaerella graminicola* and *Fusarium graminearum*.

One of the main objectives of genome sequencing of plant pathogens has been to identify genes involved in pathogenicity and to study the interactions between host and pathogen. Gene function is traditionally investigated through gene disruption studies. Functional genomics studies have been widely applied to *M. oryzae* due to its amenability to transformation and tractability of the infection process (Wilson & Talbot, 2009). For example Jeon et al. (2007) generated 21,070 *M. oryzae* mutants using an *Agrobacterium tumefaciens* mediated transformation method to study genotype-phenotype relationships. This method identified 202 new loci involved in *M. oryzae* pathogenicity. Other researchers have developed a method of gene functional analysis in *M. oryzae* using RNA interference (RNAi) (Caracuel-Rios & Talbot, 2008, Quoc Bao et al., 2008). Quoc Bao et al. (2008) used RNAi to characterise 37 putative genes involved in calcium-signalling in *M. oryzae*. The development, sporulation, appressorium formation and pathogenicity of the RNAi transformants were examined. Fifteen of the 37 genes were found to be involved with pathogenicity.

The availability of a complete genome sequence also allows genome-wide expression profiling. For example in 2006 an Affymetrix GeneChip microarray was released for

Fusarium graminearum (Gueldener et al., 2006). All of the putative genes from the complete genome sequence were included. Gene expression has now been studied in a number of situations including during infection time courses in barley (Gueldener et al., 2006) and wheat (Lysøe et al., 2011), under different culture conditions that are either deoxynivalenol mycotoxin inducing or non-inducing (Gardiner et al., 2009) and during perithecium development of the fungus (Hallen et al., 2007). Microarray studies have also been carried out with *M. oryzae*. For example Mathioni et al. (2011) evaluated gene expression during barley and rice infection in comparison to *M. oryzae* growth during *in vitro* stresses (temperature, oxidative and nutrient stresses).

Currently relatively little is known about the *Ggt*-wheat interaction. *Ggt* is not easily amenable to transformation and so far there is lack of mutants available to study gene function. Transformation of *G. graminis* was carried out using polyethylene glycol (PEG)-mediated protoplast transformation in the late 1980s and 1990s, but the transformation efficiency was particularly low and the transformants were often unstable (Freeman & Ward, 2004). More recently Park et al. (2011) successfully transformed *Ggg* by PEG-based protoplast transformation. Fungal cells were transformed to generate a β -glucuronidase (GUS) producing fungal strain. The successful transformants were stable, had similar phenotypes to wild-type and were still pathogenic. The GUS-expressing strains could be very useful for studying the infection process of *Ggg*. There is only one successful report of targeted gene disruption in *G. graminis*, used to generate avenacinase mutants of *Gga* (Bowyer et al., 1995). The avenacinase mutants were no longer able to infect oats roots but were still able to infect wheat, demonstrating that a single gene can determine host range of *Gga*. If a transformation system for gene disruption in *Ggt* could be developed and optimised in the future this would allow the opportunity to understand much more about the biology of take-all disease and the genes involved in pathogenicity. Another approach would be to test putative pathogenicity genes from the genome sequence of *Ggt* in the related species *M. oryzae*. Currently, in the absence of a reliable *Ggt* transformation system, the development and use of genome-wide expression profiling is probably the best way to begin to explore the *Ggt*-wheat interaction in greater depth.

8.6. Metagenomics

Metagenomics is the culture-independent genomic analysis of a population of microorganisms (Riesenfeld et al., 2004). Over 99% of microorganisms in many

environmental samples cannot be grown in culture, and so are not easily studied (Amann et al., 1995, Streit & Schmitz, 2004). Vogel et al. (2009) state that in the soil environment less than 0.5% microorganisms are culturable. Since the 1980s DNA based molecular methods to analyse 16S rRNA gene sequences have been used to assess microbial diversity and the taxa present in different environments without the need for prior cultivation of the microbes present (Handelsman, 2004, Streit & Schmitz, 2004). Metagenomics analyses can also be used to identify the gene content of different microbial communities and to detect novel genes. DNA is extracted from an environmental sample, cloned into a vector and transformed into a host bacterium to create metagenomic libraries. Sequence and gene function analyses can then be carried out (Handelsman, 2004). Functional analyses of metagenomic libraries, involving heterologous expression of the cloned DNA in a host and assays to screen for particular functions, have been used to identify naturally occurring antibiotic resistance genes in the soil environment (Allen et al., 2009, Donato et al., 2010, Torres-Cortes et al., 2011). Functional metagenomic analyses have also been used in a European-Union sponsored project, the METACONTROL project, to investigate disease suppressive soils and identify novel antibiotics (Courtois et al., 2003, van Elsas et al., 2008). Soils suppressive to *Rhizoctonia solani*, *Plasmodiophora brassicae* and *Fusarium* spp. were identified and screened for functional antibiotics for potential biotechnological use and control of phytopathogens. The METACONTROL project developed a range of technologies to optimise DNA extraction, the vector/host system, the functional and molecular screening method of the library clones and the analysis of results for the exploration of soil metagenomic libraries.

In addition to sequencing individual genomes of species of interest the improvement in genomics technology now allows the sequencing of whole microbial communities from environmental metagenomic libraries. Ocean microbial communities have been sequenced to compare genomic similarity and gene content in different environments (Venter et al., 2004, Rusch et al., 2007). More recently Vogel et al. (2009) have set up an international consortium to sequence the soil metagenome (International Soil Metagenome Sequencing Consortium <http://www.terragenome.org/>). This is to provide information on gene diversity and function in the soil environment. The soil environment chosen for sequencing is from the classical long term Park Grass agroecology field experiment at Rothamsted Research UK (<http://www.rothamsted.ac.uk/Content.php?Section=Resources&Page=ExperimentsGui>

de). This will provide a reference soil metagenome sequence to compare other soils with, and could potentially provide more information on ecosystem functions and microbial processes in the soil.

Metagenomic research has been applied to the *Ggt*-wheat interaction and analysis of take-all epidemics. Recently Sanguin et al. (2009) provided new insights into changes in the microbial soil community during the development of take-all decline in wheat monoculture based on 16S rRNA gene analysis. Sanguin et al. (2009) assessed changes in the whole bacterial community of rhizosphere samples collected from plots sown to wheat for 1 year (low disease), 5 years (high disease) or 10 years (decline) using a 16S r-RNA-based microarray. This study revealed that there were various changes in the composition of bacterial community at the different disease stages and that decline could be the result of more complex community-based interactions. This type of 16S r-RNA study allows microbial diversity to be assessed in different environmental situations but does not give any information about the functional role of the microorganisms. Such an approach would be useful to assess the diversity and composition of rhizosphere microbial communities in the soil underneath low and high TAB varieties at different time points during the growing season. This would provide a way of starting to characterise the low TAB phenotype and evaluating the possibility that a microbial-*Ggt* interaction is involved with the low TAB trait.

Current improvements in sequencing technology will help soil metagenomic studies allowing a greater understanding of microbial diversity and function in the soil and could potentially be very useful in understanding more about the microbial influence on *Ggt* epidemics within a single season and between the various wheat genotypes.

8.7. Future of take-all control

Resistance to *Ggt* is not currently used as a control measure for take-all of wheat. It has previously been suggested that the lack of resistance of wheat and its relatives to take-all is evidence that there has been little evolutionary selection pressure by this disease (Cook, 2003). However, evidence from this PhD suggests that the related species *T. monococcum* does show resistance against take-all and that modern hexaploid wheat varieties differ in their ability to build-up take-all inoculum in the soil. Both of these findings could be useful in future control strategies. It is not yet clear how valuable this genetic material for take-all control could be and how an integrated approach with other

cultural and chemical control strategies might work. The impacts of environmental conditions on disease severity are difficult to quantify and forecast and can vary in different areas of the world. It is evident that if environmental conditions are particularly conducive to disease then severe take-all disease will still be a problem and so the use of genetic material should be combined with other control measures. One of the cultural control strategies described in Chapter 1 is sowing second wheat crops later to increase the length of the inter-crop period and so the decline of take-all inoculum in the soil (Colbach et al., 1997, Hornby et al., 1998, Cook, 2003). However, later sown crops tend to yield less in the absence of take-all. It would be useful to explore different management strategies to investigate how cultural control methods and genetic control can be combined. For instance would it be possible to sow second wheat crops earlier after a low TAB first wheat variety.

The durability of resistance is difficult to predict but is likely to be durable due to the polygenic nature of the low TAB trait and likely polygenic nature of the *T. monococcum* resistance trait. *Ggt* is a homothallic fungus and the exchange of genetic material under field conditions is presumed to be rare (Hornby et al., 1998). The spread of *Ggt* isolates able to overcome host resistance is therefore likely to be quite slow. It would be useful to identify other sources of resistance in related species that could be combined to increase the level of resistance deployed. One of the challenges will be to maintain high inherent yields while also incorporating resistance QTLs. The sequencing of the wheat genome (and potentially in the future other related species) in combination with improvements in genomics technology to allow efficient marker assisted selection should increase the accuracy by which resistance is incorporated.

8.8. Food security

The worldwide human population is projected to reach 9 billion by 2050 and the Food and Agriculture Organisation of the United Nations (FAO) predicts that food production will need to double by 2050 to meet this demand (www.fao.org). This increase in demand in combination with climate change, land degradation, high energy prices and additional land pressure due to population growth and introduction of biofuels makes current and future food security a major challenge. As mentioned in Chapter 1 pests and diseases are estimated to reduce yields by up to 40% worldwide (Oerke, 2006), making crop diseases a significant threat to global food security (Strange & Scott, 2005, Mahmuti et al., 2009, Flood, 2010, Cook et al., 2011). In the UK plant breeding has

had a major contribution to wheat yields over the last 50 years and the average wheat yield is now over 8 tonnes per ha, an increase of nearly 4 tonnes per ha since the 1950s (Mackay et al., 2011). However, analysis of historical wheat yield data by Mackay et al. (2011) provides evidence that in the last twenty years there has been a decline in the rate of increase in wheat yields. Wheat is the dominant cereal crop in the UK and northern hemisphere and so it is essential that yield potential is increased and protected to cope with future risks to global food security. Bruce (2012) suggests that GM (Genetic Modification) technology is a powerful tool that can be used to improve crop species and protect global food security. GM technology can speed up the plant breeding process and makes possible the transfer of useful genes into wheat from more distantly related species that cannot be transferred using conventional methods. Currently the development of GM crops has been hindered by negative public opinion and the strict and expensive regulatory protocols in place (Fedoroff et al., 2010, Tester & Langridge, 2010). Current and future technological advances for both GM and non-GM approaches will be important to help provide solutions for the growing world food demand (Lucas, 2011).

The spread of new pests and diseases is one of the major challenges faced by farmers in the UK and around the world. In wheat, a particularly virulent strain (Ug99) of black stem rust (*Puccinia graminis* f. sp. *tritici*) emerged in Africa in the 1990s (Flood, 2010). Ug99 was first identified in Uganda in 1998 and has since spread throughout the Eastern African highlands and north into Yemen and Iran (Pretorius et al., 2000, Singh et al., 2006, Nazari et al., 2009, Singh et al., 2011). Races related to Ug99 have also been identified in Zimbabwe and South Africa (Singh et al., 2011). Strain Ug99 has a combination of virulence genes against the major rust resistance genes deployed in wheat making many previously stem rust resistant varieties now susceptible. Approximately 85-95% of wheat varieties throughout Africa and Asia are considered to be highly susceptible to the Ug99 strain and yield losses of up to 70% have been recorded, making Ug99 a major threat to wheat production (Flood, 2010, Singh et al., 2011).

Climate change is also expected to cause the spread of new pests and diseases and change the severity of outbreaks. North-west Europe is predicted to have a general increase in temperature with wetter winters but drier summer conditions (West et al., 2012). Already outbreaks of bluetongue virus in sheep and cattle populations

throughout Europe in the last 10 years have been linked to climate change. Outbreaks are thought to be the result of warmer temperatures allowing the vector host (*Culicoides* spp.) to expand its host range northwards (Purse et al., 2005). An outbreak across Europe in 2007 caused sheep population mortality rates of up to 50% in some areas (Maan et al., 2008). Warmer winter temperatures also increase the overwintering survival of other disease vectors such as potato aphids. This allows earlier infection of potatoes with Potato leafroll virus (PLRV) and Potato virus Y (PVY) (Robert et al., 2000). For many diseases the effects of climate change are hard to forecast. Recently West et al. (2012) carried out climate change modelling to predict the effect on various diseases of crops in the UK and north-west Europe. Based on this study it is expected that there would be a slight increase for some diseases such as Fusarium head blight (*Fusarium* spp.) but little change for other fungal wheat diseases such as Septoria leaf blotch (*Mycosphaerella graminicola*). Barnes et al. (2010) predicted that Phoma stem canker (caused by *Leptosphaeria maculans*) could cause a 10-50% reduction in oilseed rape yields under predicted climate change in the UK and would move further northwards in Scotland under the warmer temperatures. In the case of take-all drier spring and summer conditions would restrict the initial build-up of inoculum (as in the 2010 PhD trial). However as described in Chapter 1 drier summer conditions can exacerbate the effect of take-all. By contrast, warm and wet winter conditions could increase the level of inoculum build-up in the winter months and encourage a longer period of root infection in 2nd and 3rd wheat crops. Dry soil conditions at the end of the summer could increase survival of inoculum in the inter-crop period. It is not therefore very clear as to how take-all severity would be expected to change.

The emergence of new strains of pathogens resistant to pesticides is also a major challenge for farmers. In the UK the development of *Mycosphaerella graminicola* isolates (causing Septoria leaf blotch of wheat) resistant or with decreased sensitivity to previously good fungicide products has been widely documented (Clark, 2006, McCartney et al., 2007, Stammler & Semar, 2011). Combined with the introduction of EU regulations (Directive 2009/128/EC) restricting the use of some fungicide products, the use of durable genetic resistance to pathogens and pests will become ever more important.

If global yields of wheat and other crop species are to increase it is also important that many traits including yield itself, nutrient use, water use and tolerance to abiotic and

biotic stresses are enhanced through plant breeding. Improvements in crop agronomy will also be important. Functional root systems need to be maintained or improved for efficient nutrient and water use in different environments and also to decrease the potential for environmental pollution caused by run-off of unused fertiliser in the soil (de Dorlodot et al., 2007, Ehdai et al., 2010, Richards et al., 2010, Chochois et al., 2012, Ren et al., 2012).

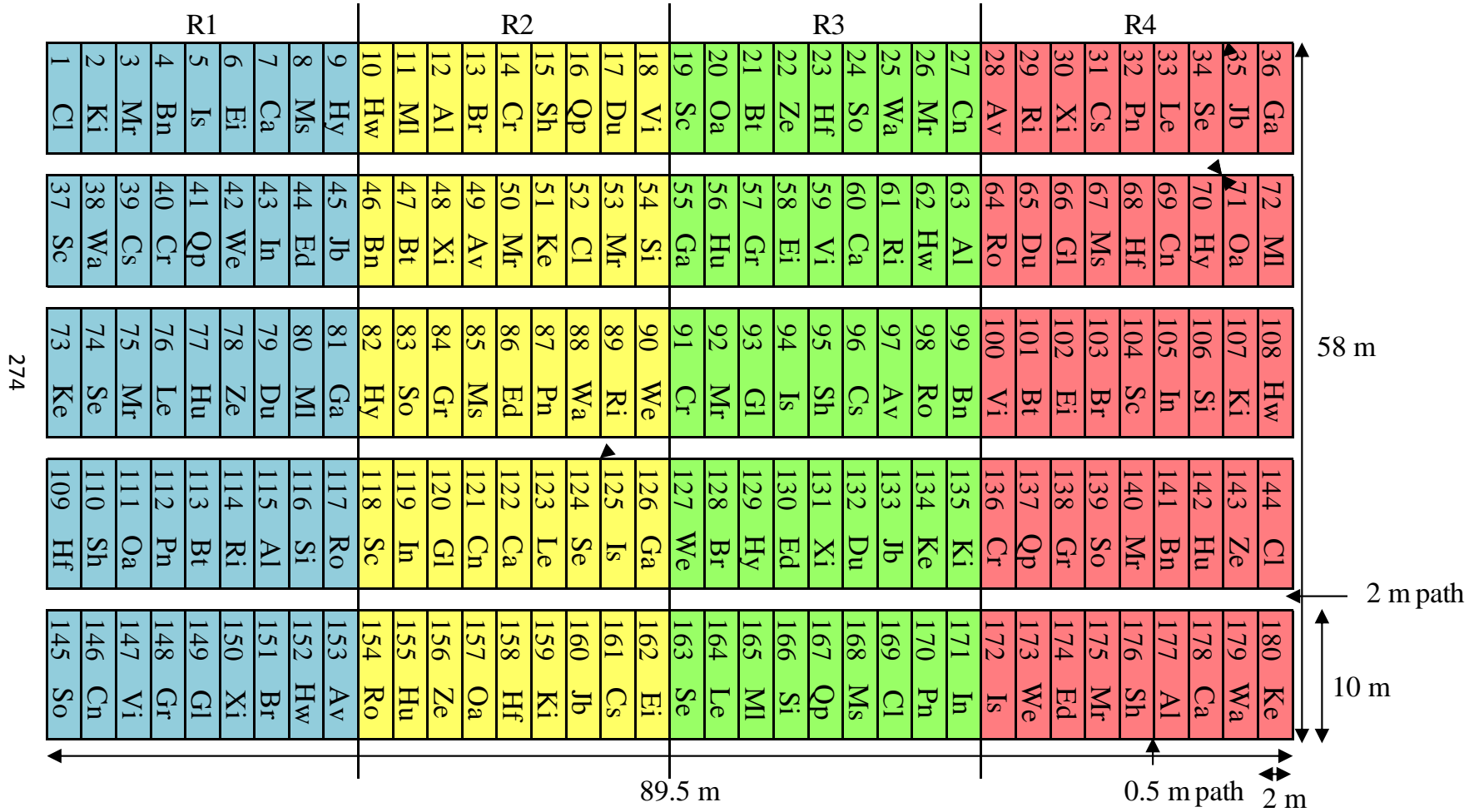
APPENDICES

Appendix 3.1. Elite winter wheat varieties and inoculum build-up field plans 2009-2011

Field trial code: 2009/R/WW/916

Field: New Zealand

Treatments: 45 winter wheat varieties x 4 reps

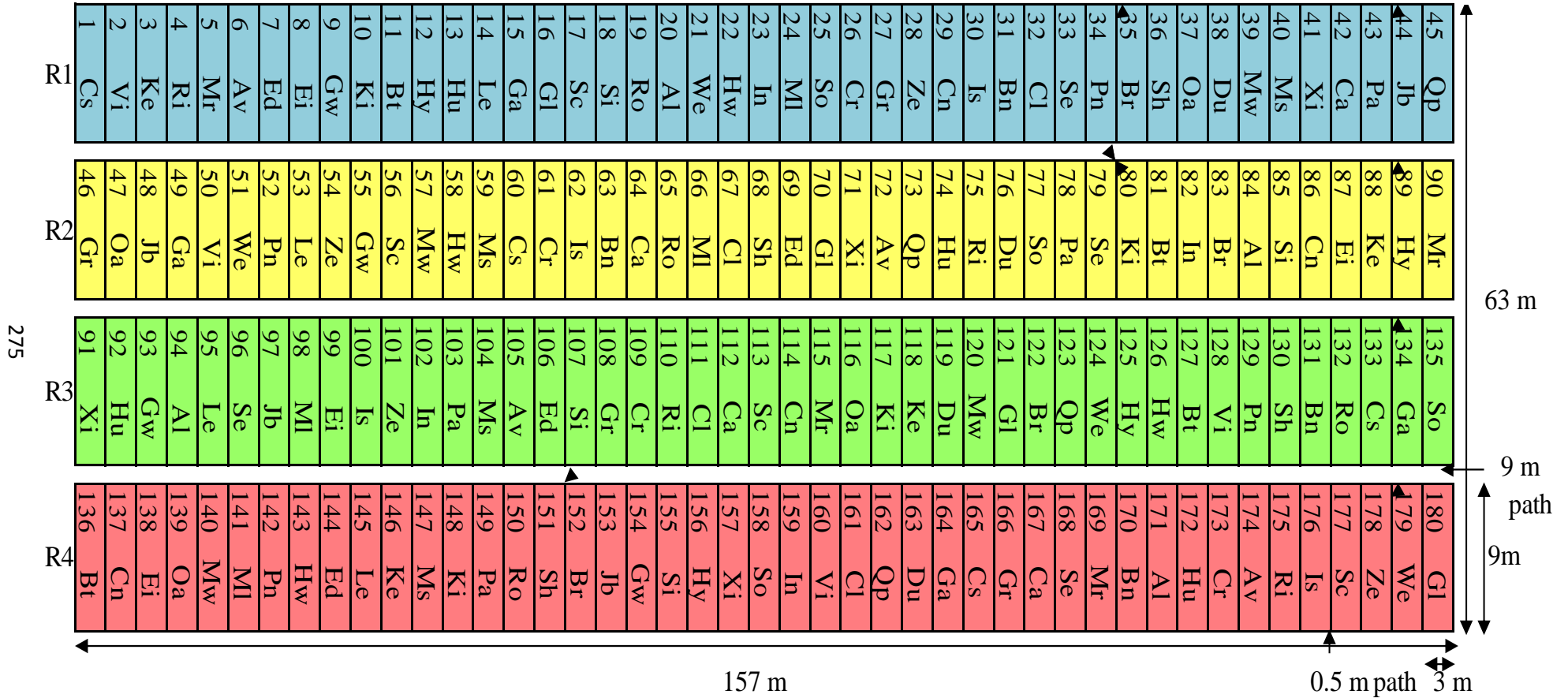


Appendix 3.1. Elite winter wheat varieties and inoculum build-up field plans 2009-2011 (Continued)

Field trial code: 2010/R/WW/1032

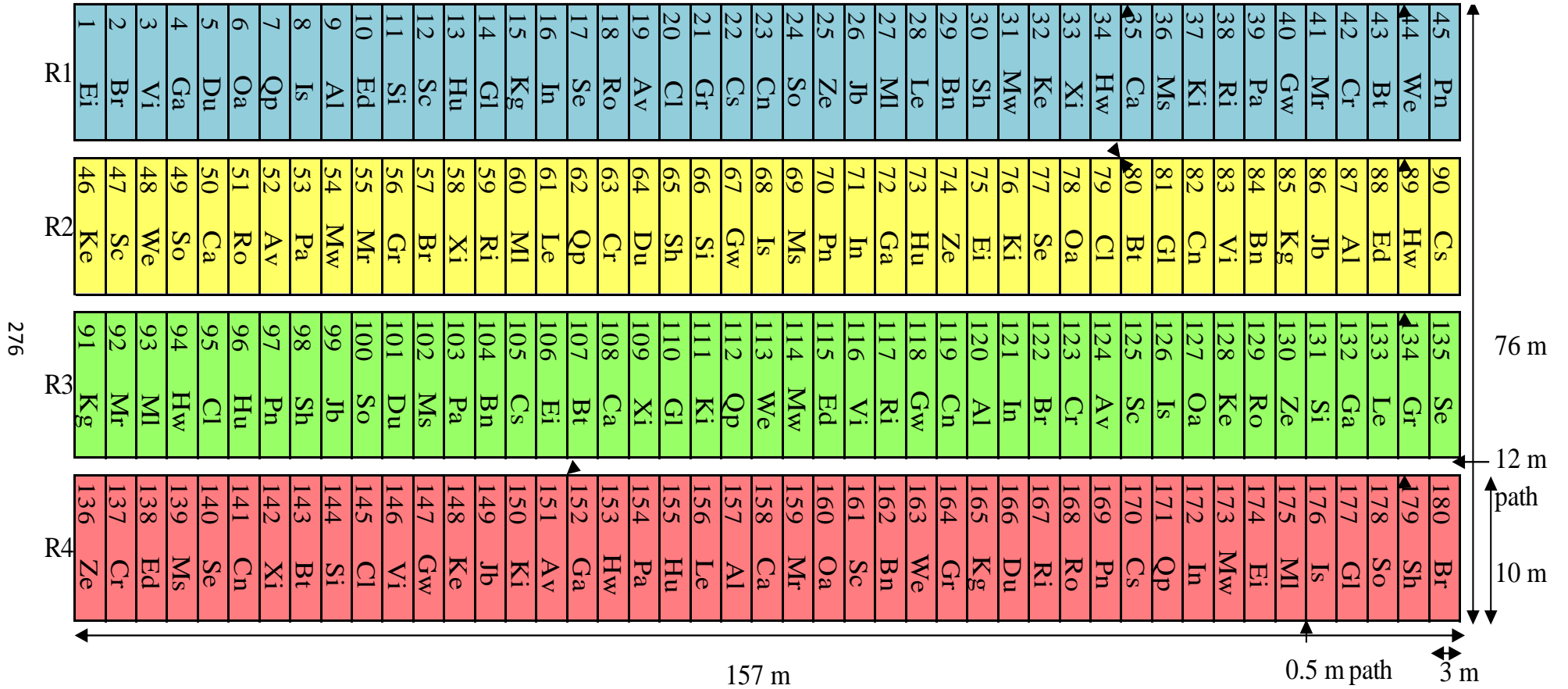
Field: Great Knott 1

Treatments: 45 winter wheat varieties x 4 reps



Appendix 3.1. Elite winter wheat varieties and inoculum build-up field plans 2009-2011 (Continued)

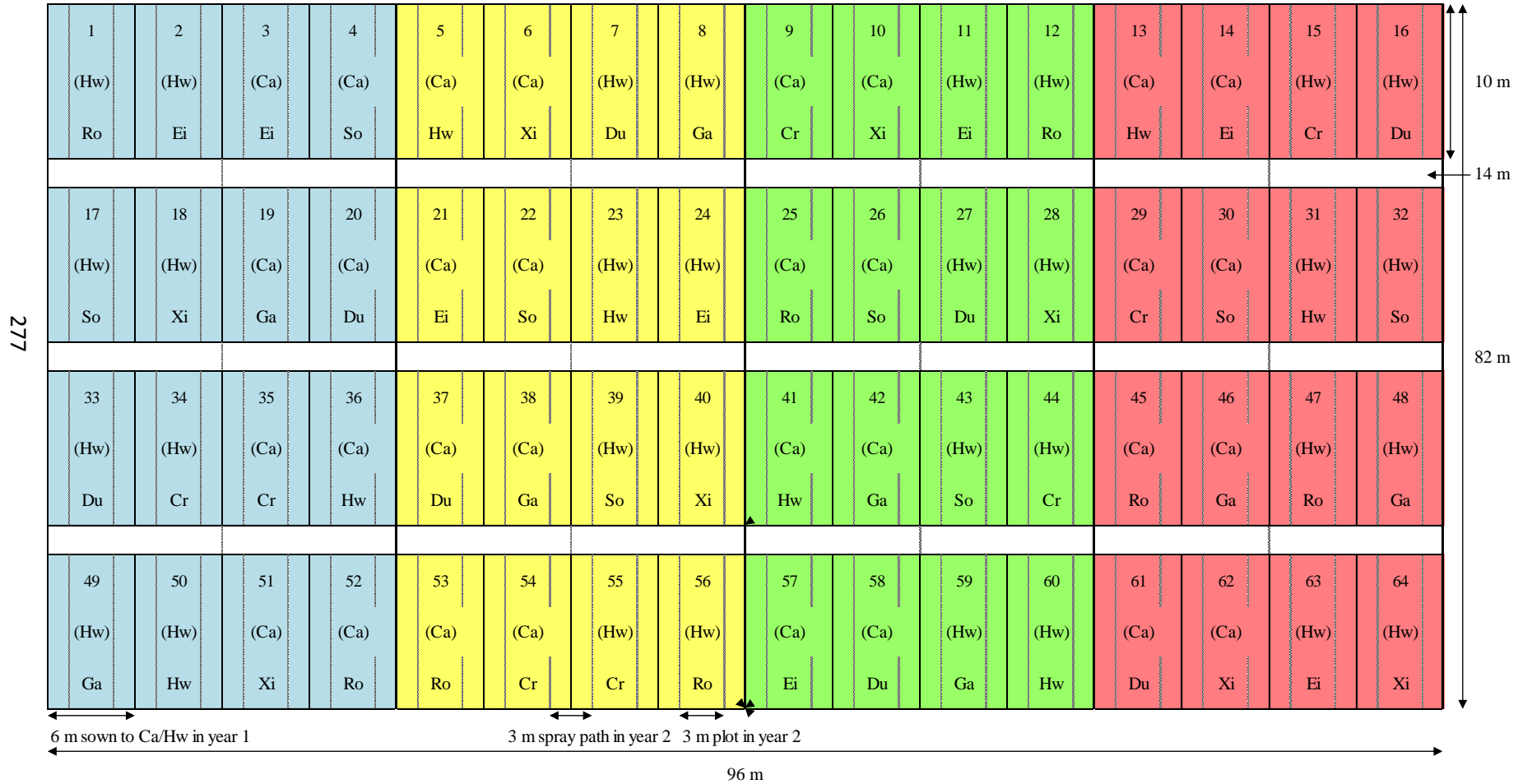
Field trial code: 2011/R/WW/1115 Field: Pastures Treatments: 45 winter wheat varieties x 4 reps



Appendix 3.2. Rotation trials 1 (harvest years 2009-2010) and 2 (harvest years 2010-2011)

Field trial code: Rotation trial 1, Year 1: 2009/R/CS/688, Year 2: 2010/R/CS/688 Field: Great Knott 3

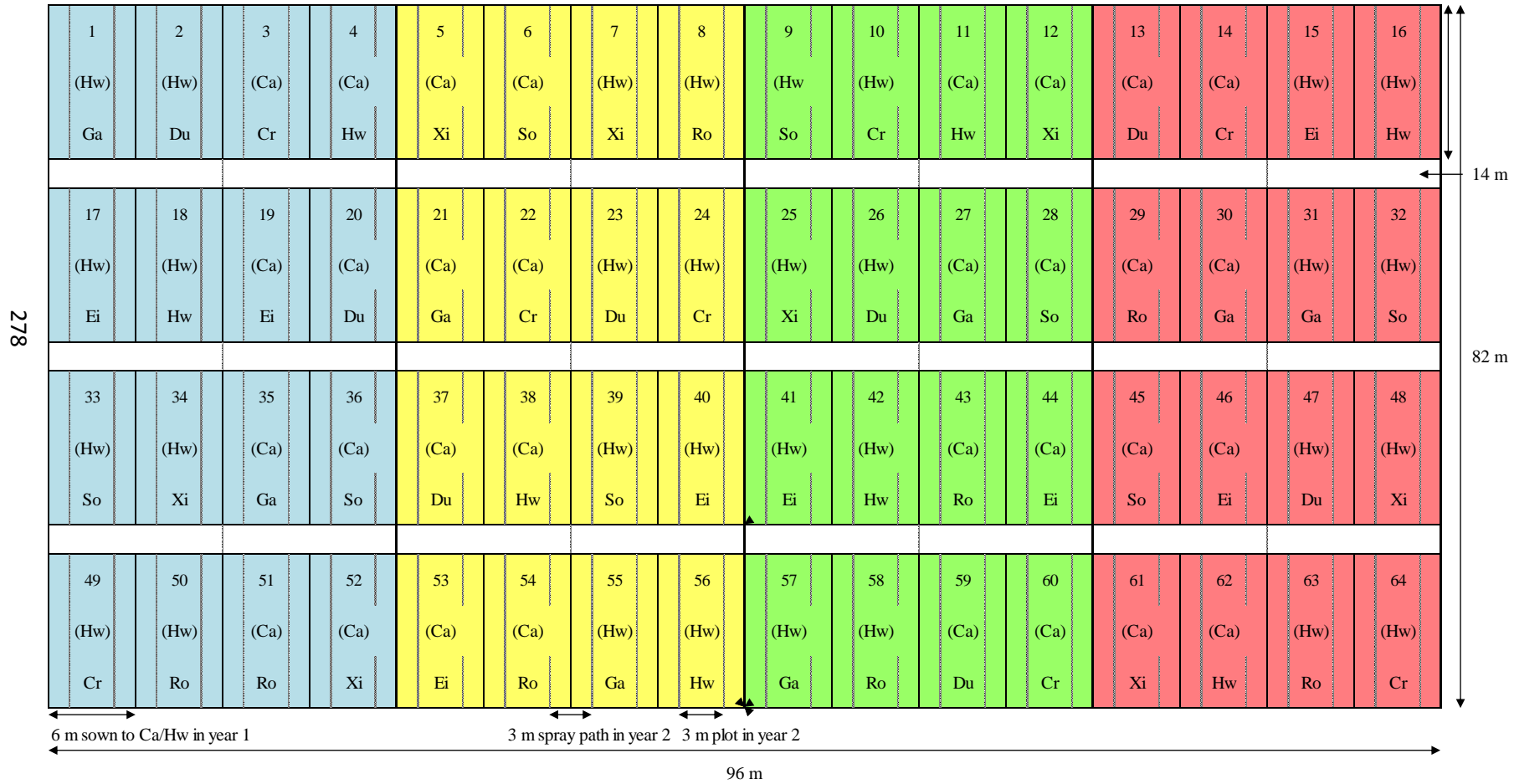
Treatments: Year 1 (in brackets) – Hereward (Hw) and Cadenza (Ca) x 4 reps Year 2 - 8 varieties x 8 reps



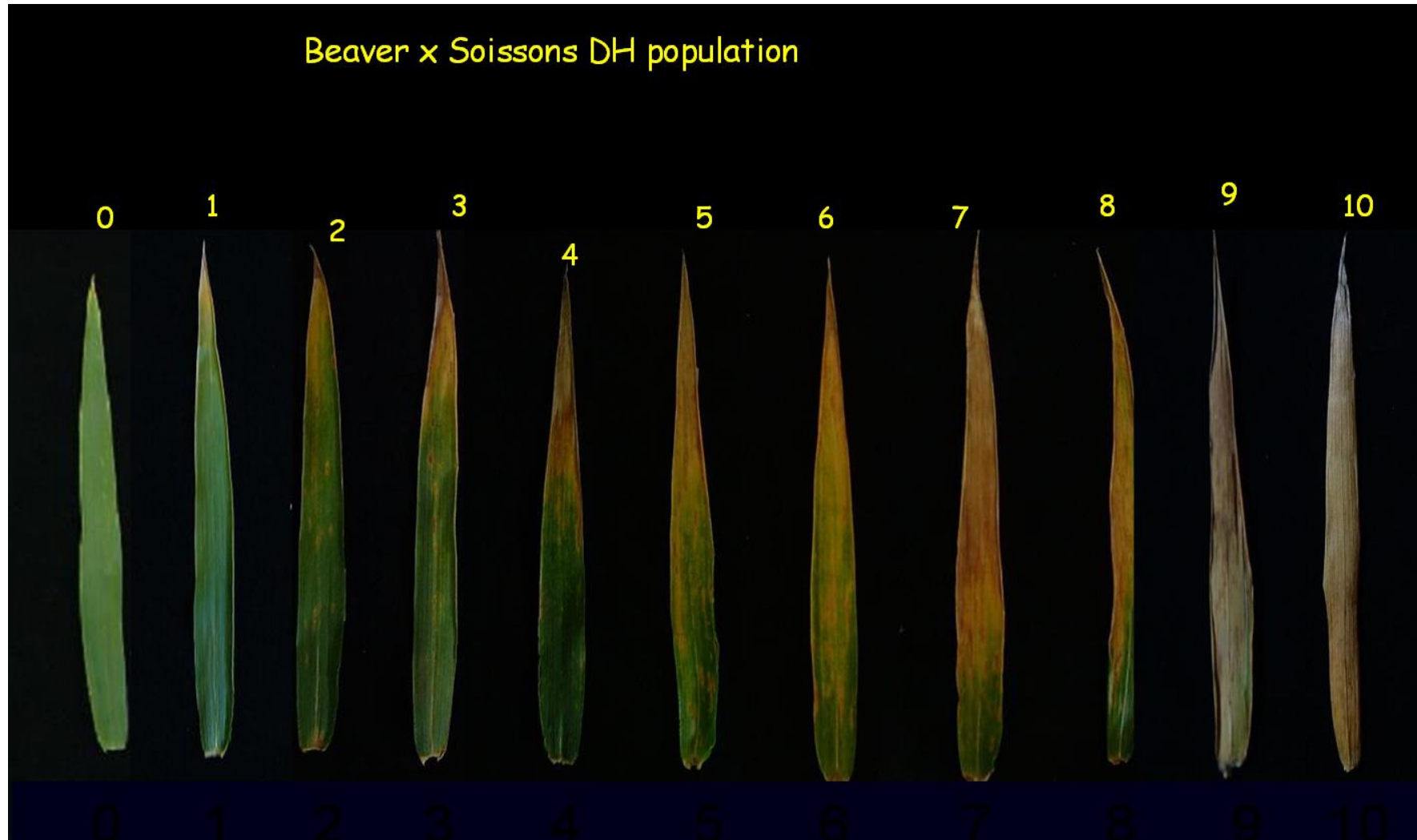
Appendix 3.2. Rotation trials 1 (harvest years 2009-2010) and 2 (harvest years 2010-2011) (Continued)

Field trial code: Rotation trial 2, Year 1: 2010/R/CS/706, Year 2: 2011/R/CS/706 Field: Great Knott 1

Treatments: Year 1 (in brackets) – Hereward (Hw) and Cadenza (Ca) x 4 reps Year 2 - 8 varieties x 8 reps



Appendix 3.3. Visual flag leaf senescence scoring



Appendix 4.1. Variety numbering in Li-Cor gel analysis

Code	Variety	Code	Variety
1	Alchemy	25	Kipling
2	Avalon	26	Lear
3	Bantam	27	Malacca
4	Battalion	28	Marksman
5	Brompton	29	Mascot
6	Cadenza	30	Monty
7	Cassius	31	Oakley
8	Claire	32	Panorama
9	Conqueror	33	Qplus
10	Cordiale	34	Riband
11	Duxford	35	Robigus
12	Edmunds	36	Scout
13	Einstein	37	Sherborne
14	Gallant	38	Shogun
15	Gladiator	39	Soissons
16	Grafton	40	Solstice
17	Hereford	41	Viscount
18	Hereward	42	Walpole
19	Humber	43	Welford
20	Hyperion	44	Xi19
21	Invicta	45	Zebedee
22	Istabraq	46	Paragon
23	JB Diego	47	Limerick
24	Ketchum		

Appendix 4.2. Preparation of DNA extraction buffers

DNA extraction buffer, pH 9.5, 1 l

12.1 g Trizma Base

74.55 g Potassium chloride (KCl)

20 ml 0.5M Ethylenediaminetetraacetic acid (EDTA)

Make up to 1000 ml with sterile distilled H₂O, adjust pH to 9.5 and store at room temperature.

On the day of use add the following:

7.5 g per litre Polyvinylpyrrolidone 40 (PVP-40)

3.6 g per litre Sodium Bisulphite

5M Potassium acetate, pH 5.8, 0.5 l

147 g Potassium acetate (KOAc)

57.5 ml Glacial Acetic Acid

Make up to 500 ml with sterile distilled H₂O, adjust pH to 5.8 using potassium hydroxide pellets, autoclave and store at room temperature.

Appendix 4.3. Li-Cor gel preparation (protocol from Kostya Kanyuka; Applications manual Model 4300 DNA Analyser, Li-Cor Biosciences)

1. Clean 25 cm glass gel plates three times with sterile distilled water and three times with 70% ethanol using kimwipe tissue paper on the side of the plates that will contact the gel.
2. Assemble gel plates and 0.25mm gel spacers. Place the left and right rail assemblies over the plate edge and tighten until finger tight.
3. Mix 16 ml Gel solution (Li-Cor) with 4 ml Gel buffer (Li-Cor), then add 160 μ l APS (Ammonium Persulfate Solution- provides a source of free radicals for polymerisation of the gel) and mix well.
4. Use a large syringe to pour the gel. Keep the gel plates on a gentle slope using the casting stand and inject the gel at a steady pace moving the syringe from side to side. Tap (using fingers) the gel plate ahead of the gel solution to avoid any bubbles forming in the gel. Once the gel reaches the bottom of the plates quickly lay the gel plates down flat.
5. Add a small amount of gel solution around the top where the comb is to be inserted then insert the sharktooth comb upside down (flat side down). Place the casting plate over the gel and tighten.
6. Leave the gel to polymerize for 45 minutes.
7. Remove the casting plate and comb (pour sterile distilled water over the top of gel/comb area to help when removing).
8. Use 3MM Whatman paper strips to remove any remains of the polymerised gel from between the glass plates in the top region where the comb is to be inserted.
9. Insert the sharktooth comb (48 well).
10. Clean the front and back plates three times with sterile distilled water and three times with 70% ethanol using kimwipe tissue paper.
11. Place the gel in the Li-Cor 4300 gel machine, fill the buffer tanks with 10 x TBE buffer and connect the power cables (see Li-Cor Applications manual for full instructions).
12. Pre-run the gel for 25 minutes.
13. Just before loading samples flush the comb area with TBE buffer using the syringe to get rid of any particulate matter.

Appendix 4.4. SSR analyses

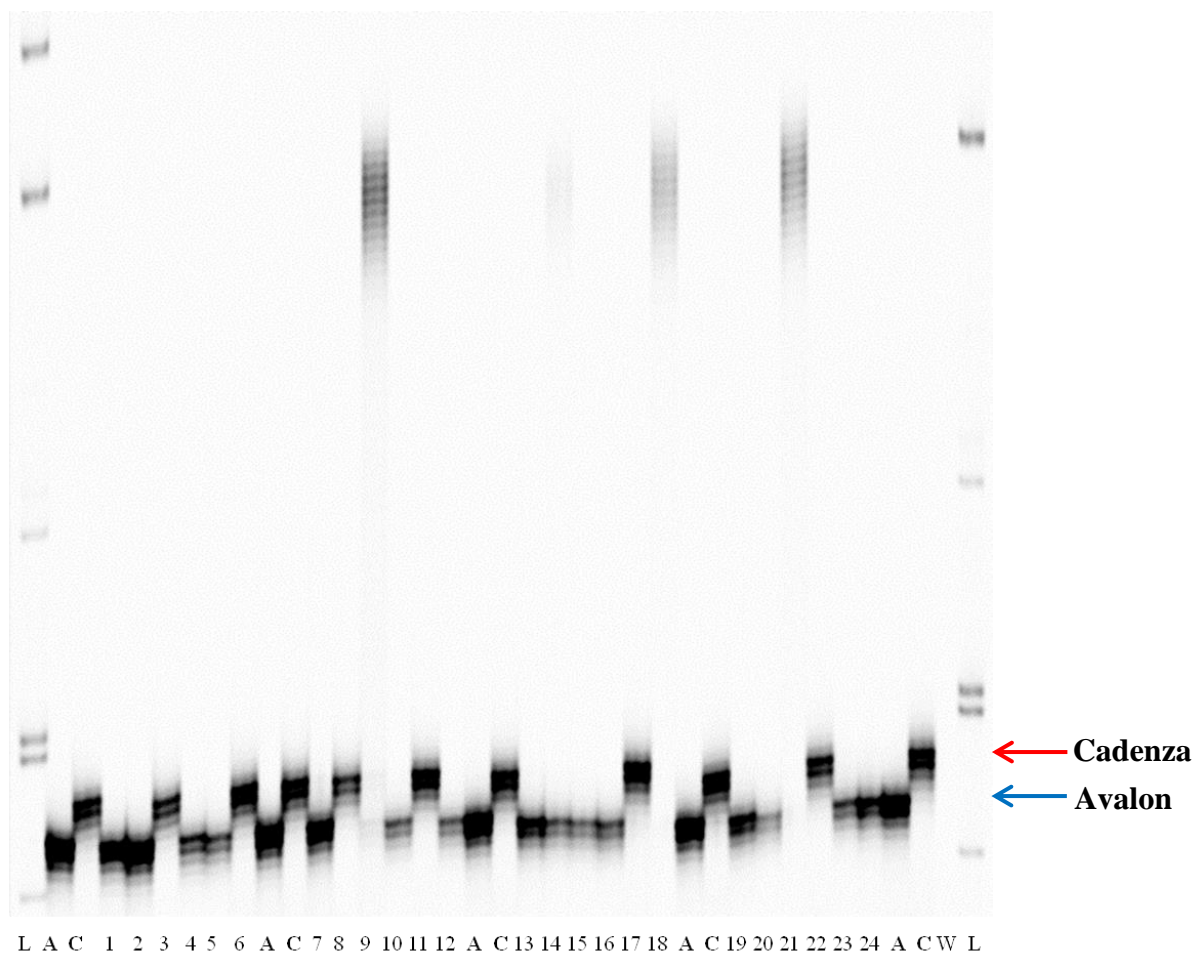


Figure A. Marker alleles at XM001 on chromosome A. Varieties are numbered as shown in appendix 4.1; varieties 1-24 this page and varieties 25-47 next page. Control lane A = Avalon, C = Cadenza. L = size ladder. W = water control. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

Appendix 4.4. SSR analyses (Continued)

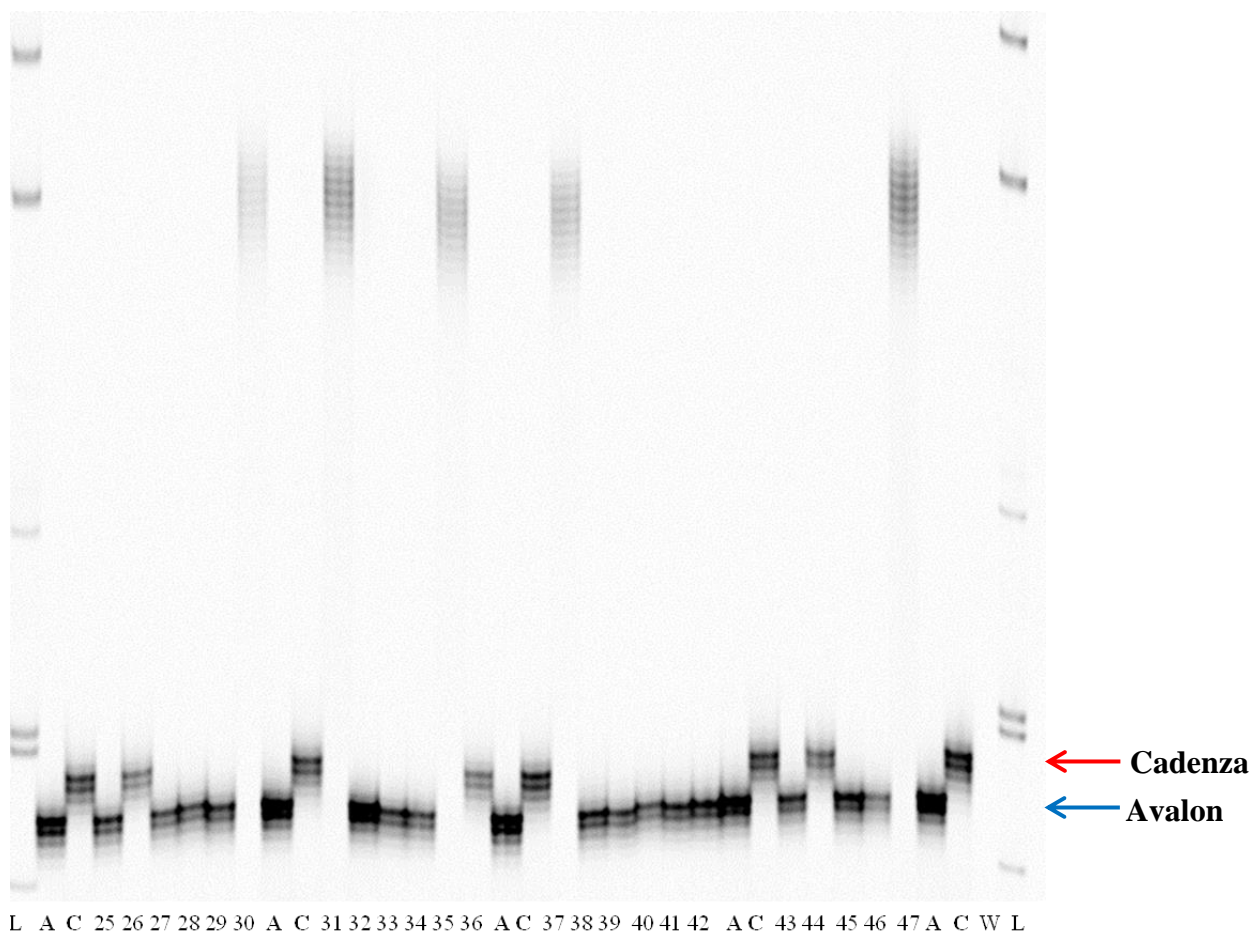


Figure B. Marker alleles at XM001 on chromosome A. Varieties are numbered as shown in appendix 4.1; varieties 1-24 previous page and varieties 25-47 this page. Control lane A = Avalon, C = Cadenza. L = size ladder. W = water control. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

Appendix 4.4. SSR analyses (Continued)

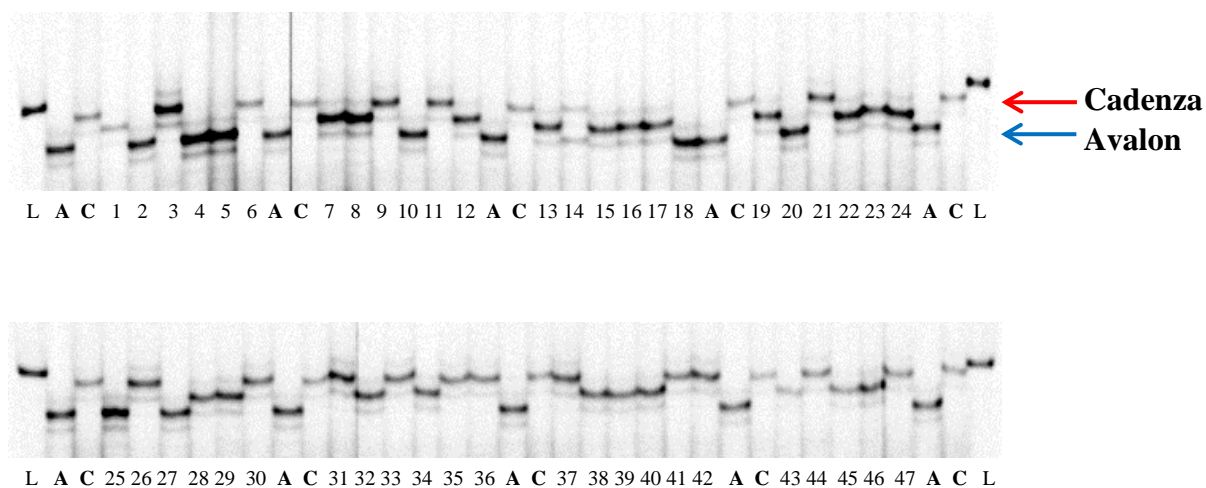


Figure C. Marker alleles at XM002 on chromosome A. Varieties are numbered as shown in appendix 4.1. Control lane A = Avalon, C = Cadenza. L = size ladder. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

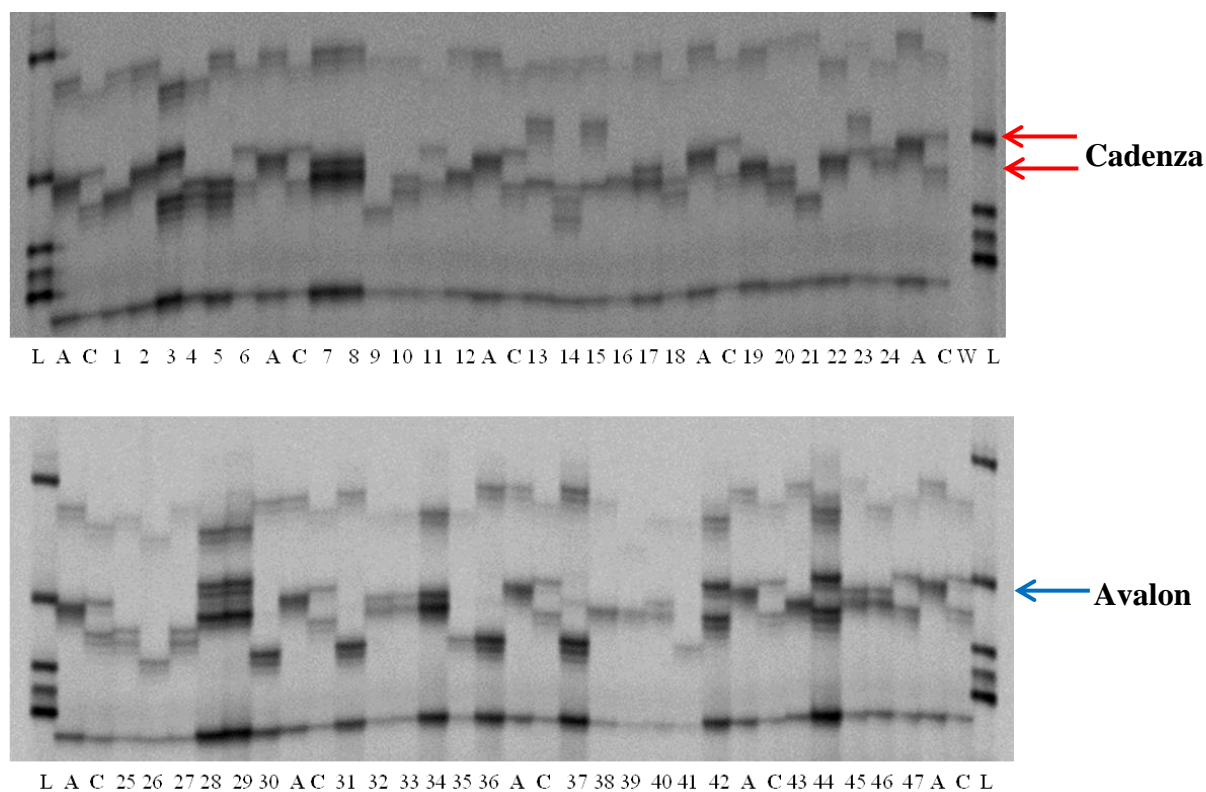


Figure D. Marker alleles at XM003 on chromosome A. Varieties are numbered as shown in appendix 4.1. Control lane A = Avalon, C = Cadenza. L = size ladder. W = water control. Arrows indicate the position of diagnostic bands for Cadenza and Avalon. Cadenza = two PCR bands.

Appendix 4.4. SSR analyses (Continued)

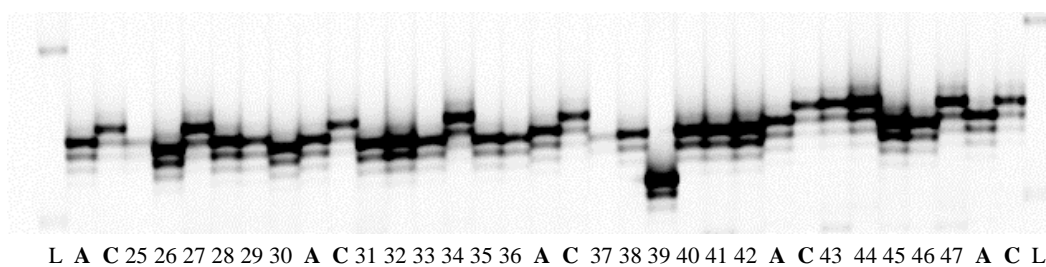
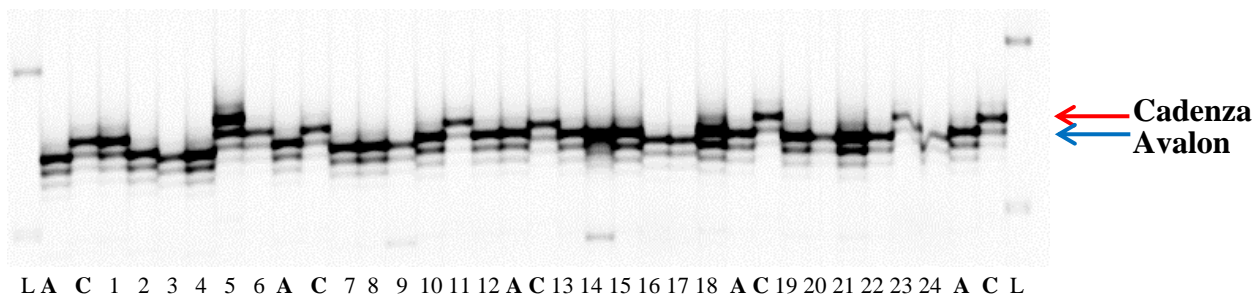


Figure E. Marker alleles at XM004 on chromosome location A. Varieties are numbered as shown in appendix 4.1. Control lane A = Avalon, C = Cadenza. L = size ladder. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

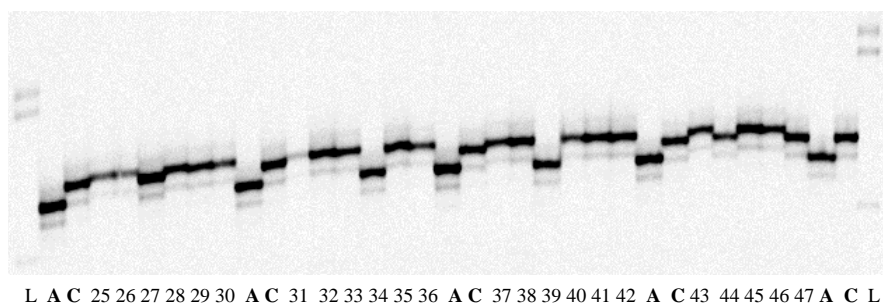
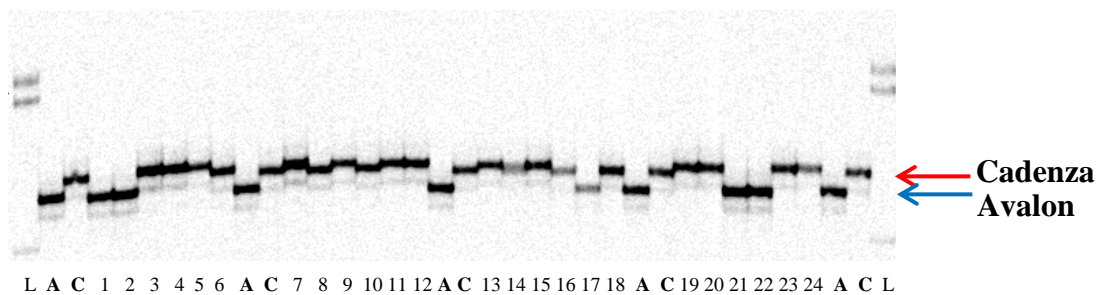


Figure F. Marker alleles at XM005 on chromosome B. Varieties are numbered as shown in appendix 4.1. Control lane A = Avalon, C = Cadenza. L = size ladder. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

Appendix 4.4. SSR analyses (Continued)

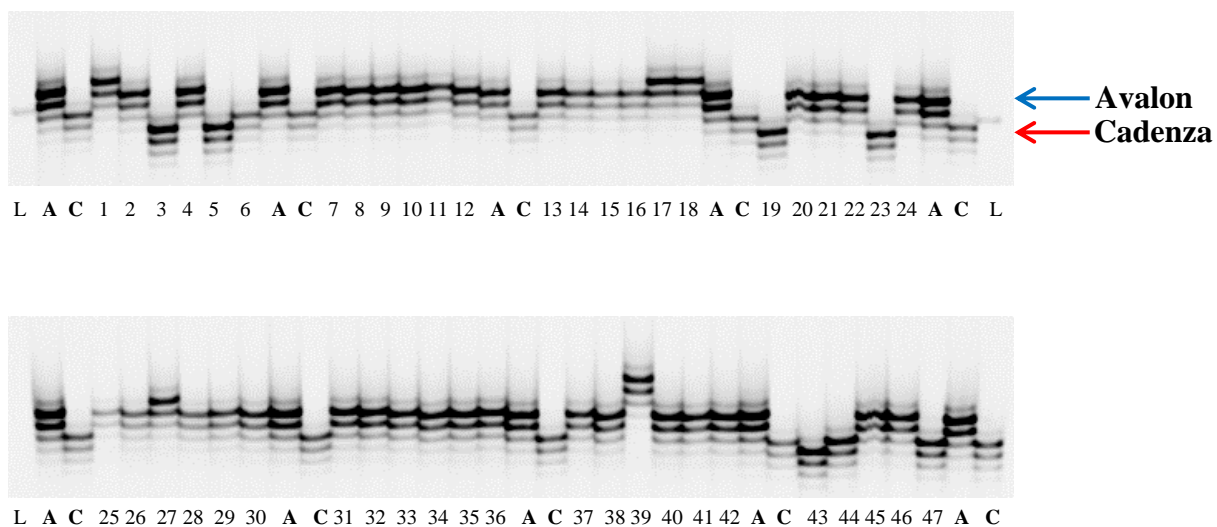


Figure G. Marker alleles at XM006 on chromosome B. Varieties are numbered as shown in appendix 4.1. Control lane A = Avalon, C = Cadenza. L = size ladder. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

Appendix 4.4. SSR analyses (Continued)

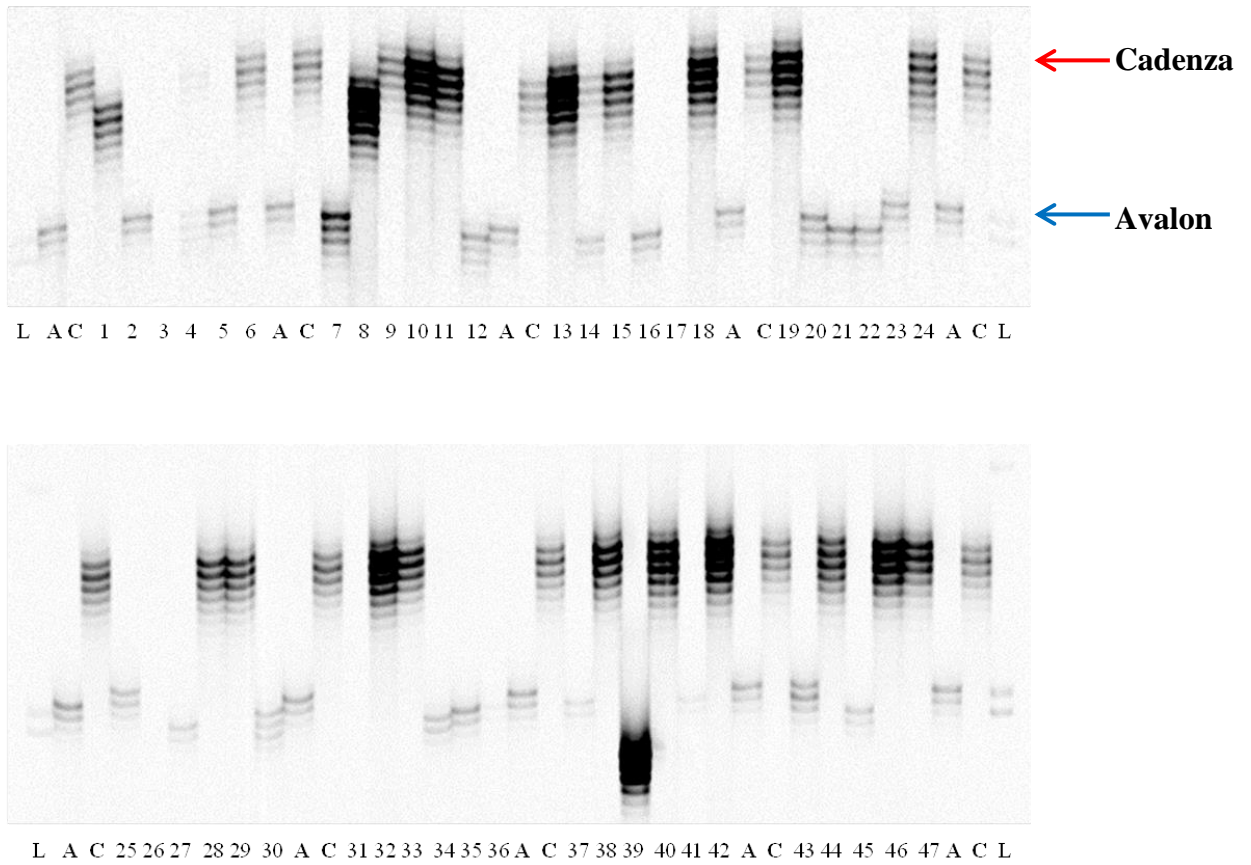


Figure H. Marker alleles at XM007 on chromosome B. Varieties are numbered as shown in appendix 4.1. Control lane A = Avalon, C = Cadenza. L = size ladder. Arrows indicate the positions of diagnostic bands for Cadenza and Avalon. Bands for varieties 3, 4, 14, 17, 26, 31, 36 and 41 very faint on gel. Re-run at lower dilution as shown in Figure I (next page).

Appendix 4.4. SSR analyses (Continued)

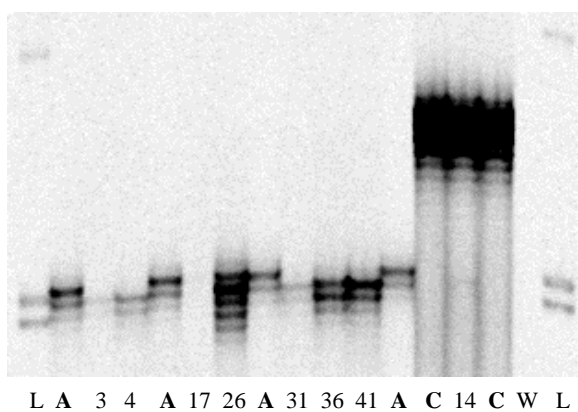
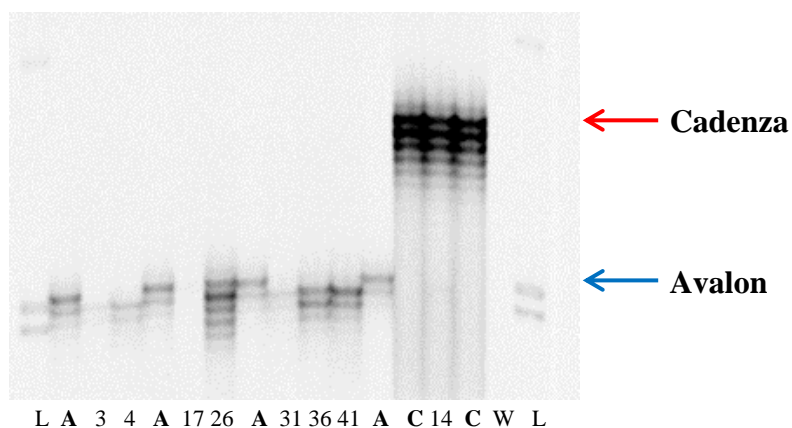


Figure I. Marker alleles at XM007 on chromosome B. Eight samples re-run at lower dilution to visualise banding patterns. Top picture = low contrast to see higher bands clearly. Bottom picture = high contrast to see lower bands clearly. Varieties are numbered as shown in appendix 4.1. Control lane A = Avalon, C = Cadenza. L = size ladder. W = water control. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

Appendix 4.4. SSR analyses (Continued)

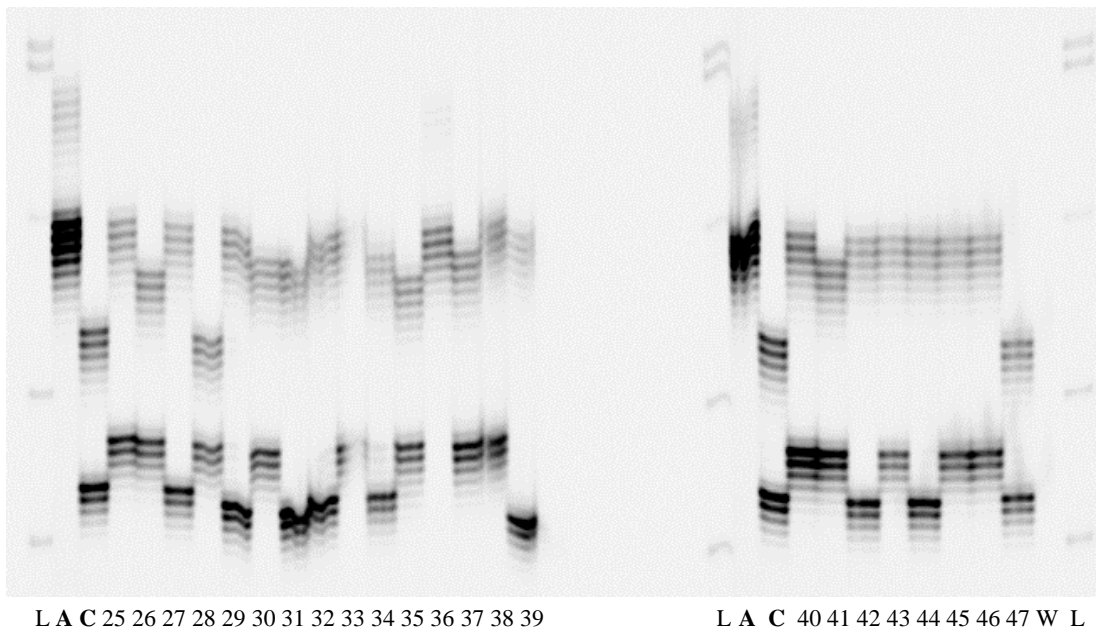
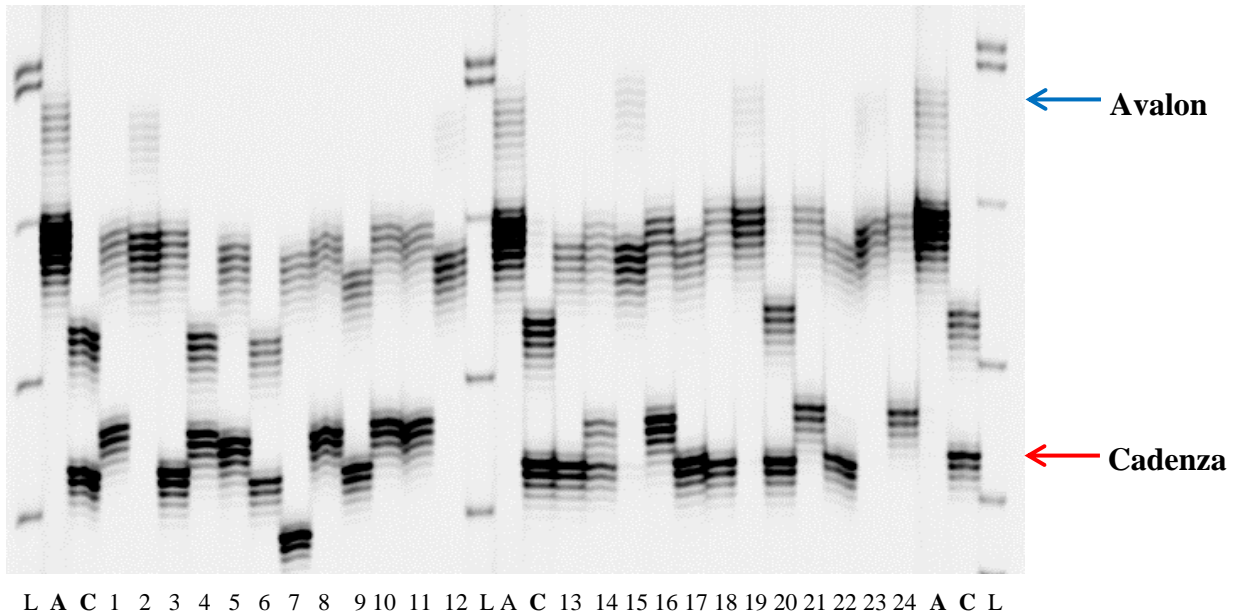


Figure J. Marker alleles at XM008 on chromosome B. Varieties are numbered as shown in appendix 4.1. Control lane A = Avalon, C = Cadenza. L = size ladder. W = water control. Arrows indicate the position of diagnostic bands for Cadenza and Avalon. Band sizes difficult to discriminate so samples re-run on further gels shown in Figure K (next page).

Appendix 4.4. SSR analyses (Continued)

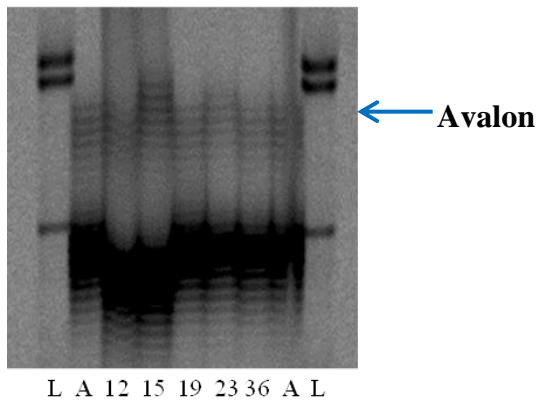
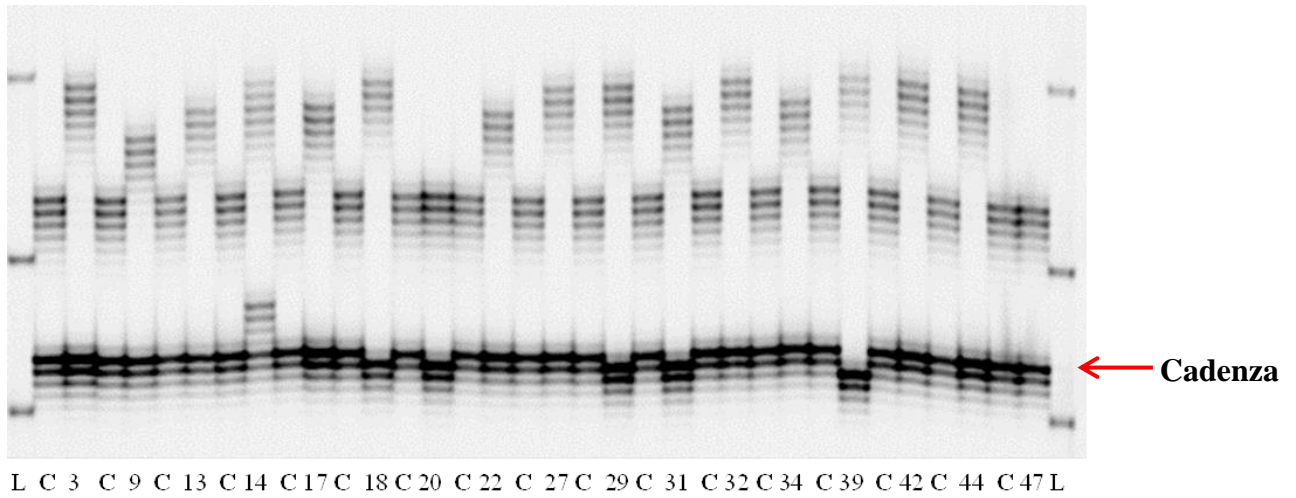


Figure K. Marker alleles at XM008 on chromosome B re-run to accurately score varieties. Varieties are numbered as shown in appendix 4.1. Control lane A = Avalon, C = Cadenza. L = size ladder. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

Appendix 4.4. SSR analyses (Continued)

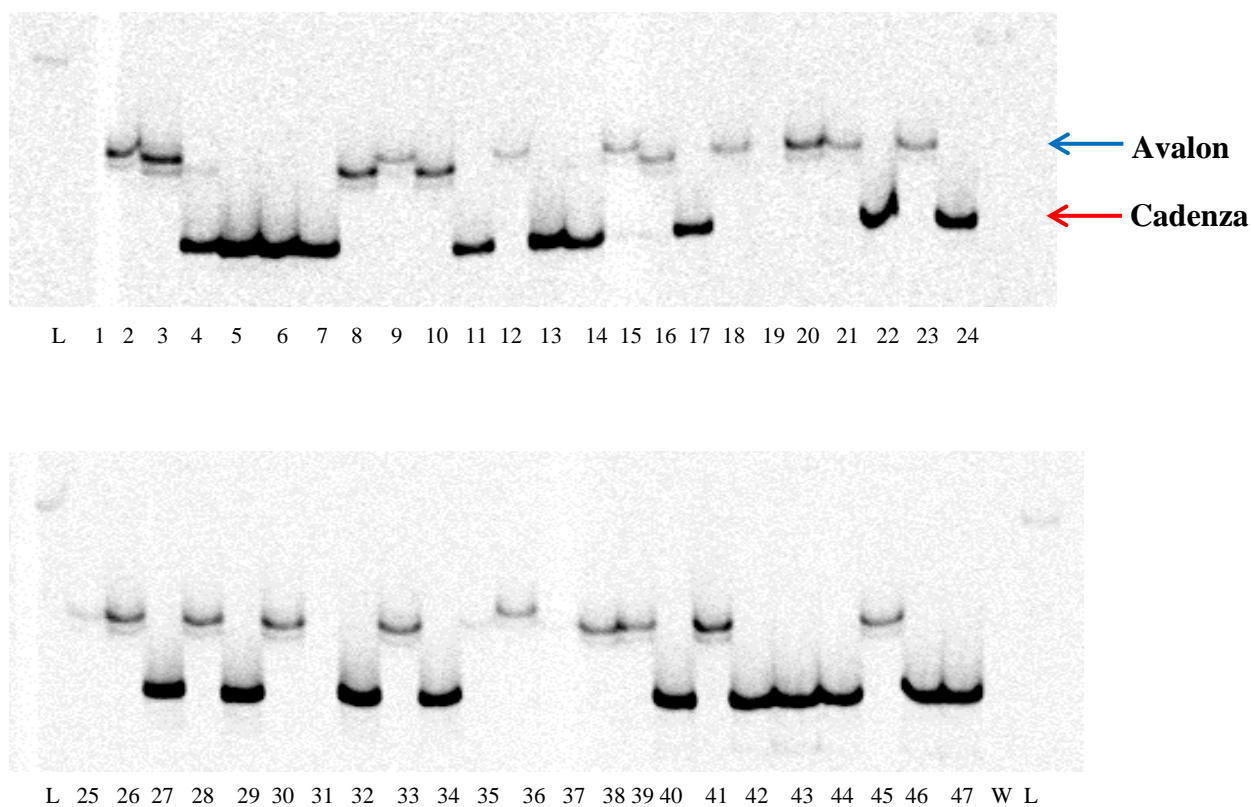


Figure L. Marker alleles at XM009 on chromosome B. Varieties are numbered as shown in appendix 4.1. L = size ladder. W = water control. Arrows indicate the position of diagnostic bands for Cadenza and Avalon. No PCR product for some samples, new PCR carried out and samples re-run with Avalon and Cadenza controls as shown in Figure M (next page).

Appendix 4.4. SSR analyses (Continued)

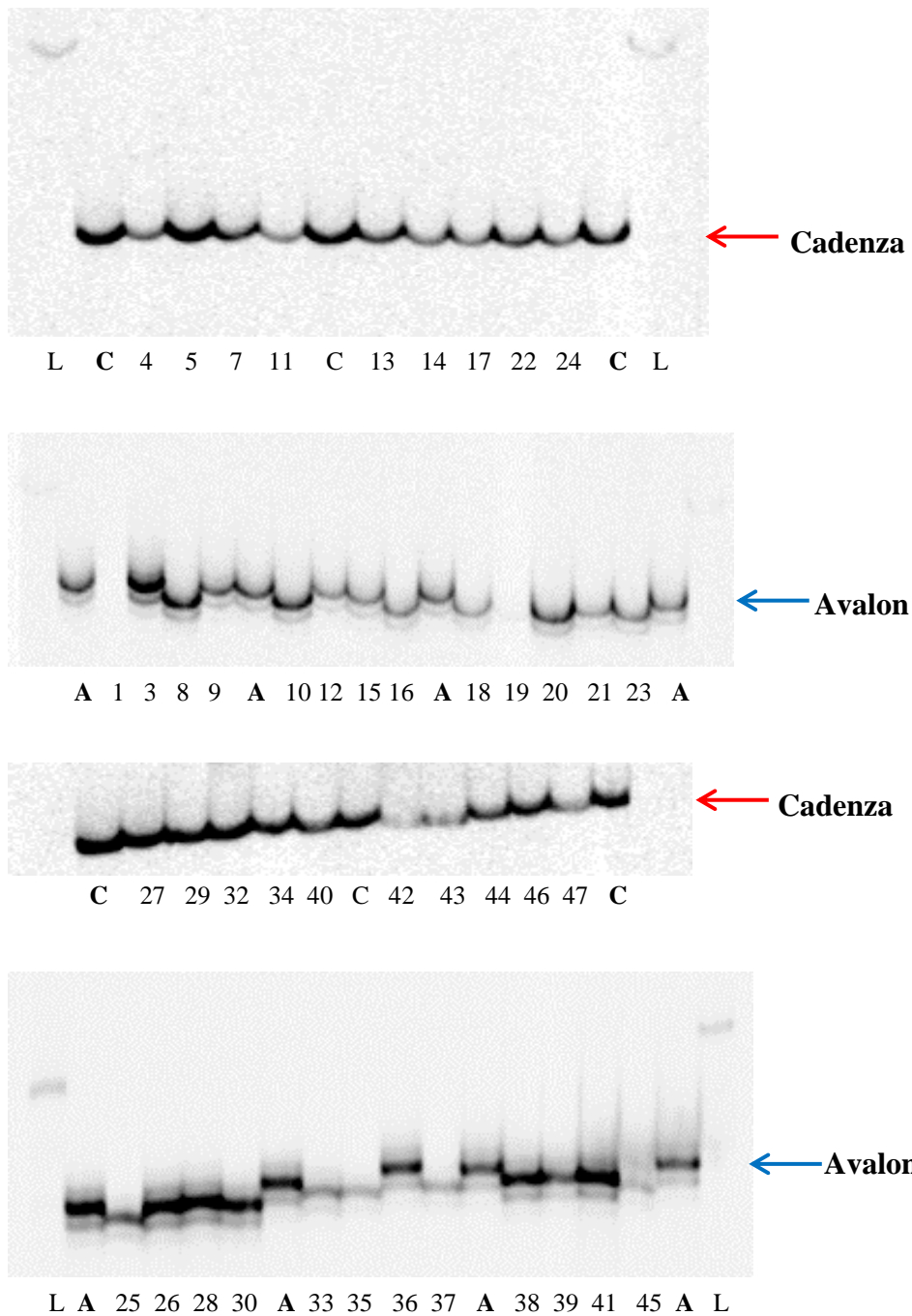


Figure M. Marker alleles at XM009 on chromosome B re-run to accurately score varieties. Varieties are numbered as shown in appendix 4.1. Control lane A = Avalon, C = Cadenza. L = size ladder. Arrows indicate the position of diagnostic bands for Cadenza and Avalon.

Appendix 5.1. DNA extraction buffer

DNA extraction buffer

20 ml 2x TEN

20 ml 2% Sodium dodecyl sulfate (SDS)

2 ml 1% β - mercaptoethanol

0.84 g Polyvinylpyrrolidone (PVP)

0.046 g Phenanthroline monohydrate

2x TEN (500ml)

465 ml distilled water

6.06 g 2-Amino-2-(hydroxymethyl)-1,3-propanediol (Tris base)

0.37 g Ethylenediamine tetraacetic acid disodium salt (Na₂EDTA)

8.77 g Sodium chloride (NaCl)

(adjusted to pH 7.2 with hydrochloric acid and autoclaved)

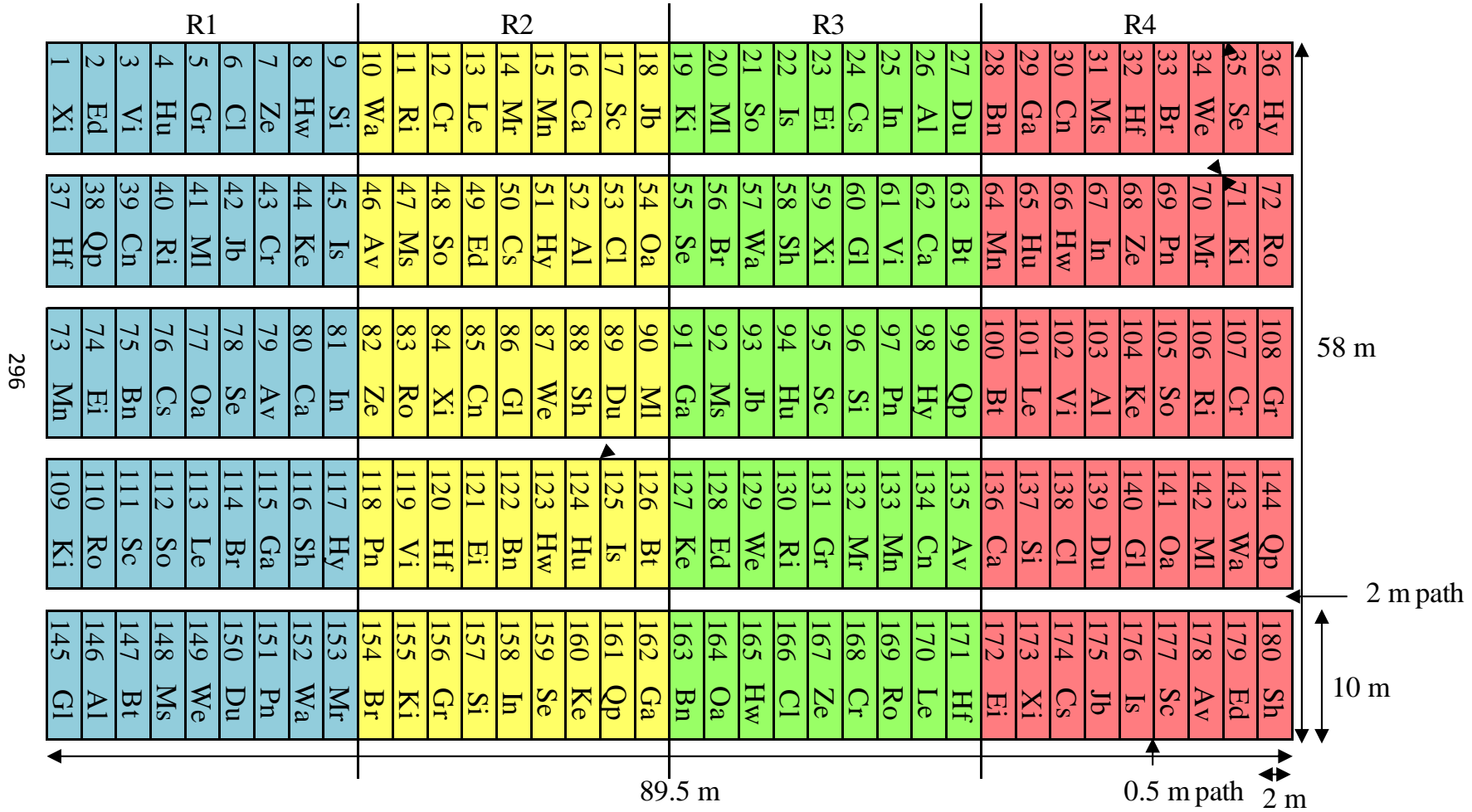
Appendix 5.2. *Ggt* isolate collection used in PhD study

Isolate ¹	Origin	Year of isolation
92·15·4A	Rothamsted, UK	1992
99S9·4B	Peterborough, UK	1999
BC01	Rothamsted, UK	2008
BC02	Rothamsted, UK	2008
BC03	Rothamsted, UK	2008
BC04	Rothamsted, UK	2008
BC05	Rothamsted, UK	2008
BC06	Rothamsted, UK	2008
BC07	Rothamsted, UK	2008
BC08	Rothamsted, UK	2008
BC09	Rothamsted, UK	2008
BC10	Rothamsted, UK	2008
BC11	Rothamsted, UK	2008
BC12	Rothamsted, UK	2008
BC13	Rothamsted, UK	2008
BC14	Rothamsted, UK	2008
BC15	Rothamsted, UK	2008
BC16	Rothamsted, UK	2008
BC17	Rothamsted, UK	2008
BC18	Rothamsted, UK	2008
BC19	Rothamsted, UK	2008
BC20	Rothamsted, UK	2008
BC21	Rothamsted, UK	2008
BC22	Rothamsted, UK	2008
BC23	Rothamsted, UK	2008
BC24	Rothamsted, UK	2008
BC25	Rothamsted, UK	2008
BC26	Rothamsted, UK	2008
BC27	Rothamsted, UK	2008
BC28	Rothamsted, UK	2008
BC29	Rothamsted, UK	2008
BC30	Rothamsted, UK	2008
BC31	Rothamsted, UK	2008
BC32	Rothamsted, UK	2008
BC33	Rothamsted, UK	2008
BC34	Rothamsted, UK	2008
BC35	Rothamsted, UK	2008
BC36	Rothamsted, UK	2008
BC37	Rothamsted, UK	2008
BC38	Rothamsted, UK	2008
BC39	Rothamsted, UK	2008
BC40	Rothamsted, UK	2008

¹ Isolates 92·15·4A and 99S9·4B were obtained from E. Ward, Rothamsted Research, originally from the culture collection of G.L. Bateman, Rothamsted Research. Isolates BC01-BC40 were isolated from soil bioassay plants grown in soil from Bones Close during this project.

Appendix 6.1. Elite winter wheat varieties and resistance to take-all field plans 2009-2011

Field trial code: 2009/R/WW/917 Field: Stackyard Treatments: 45 winter wheat varieties x 4 reps

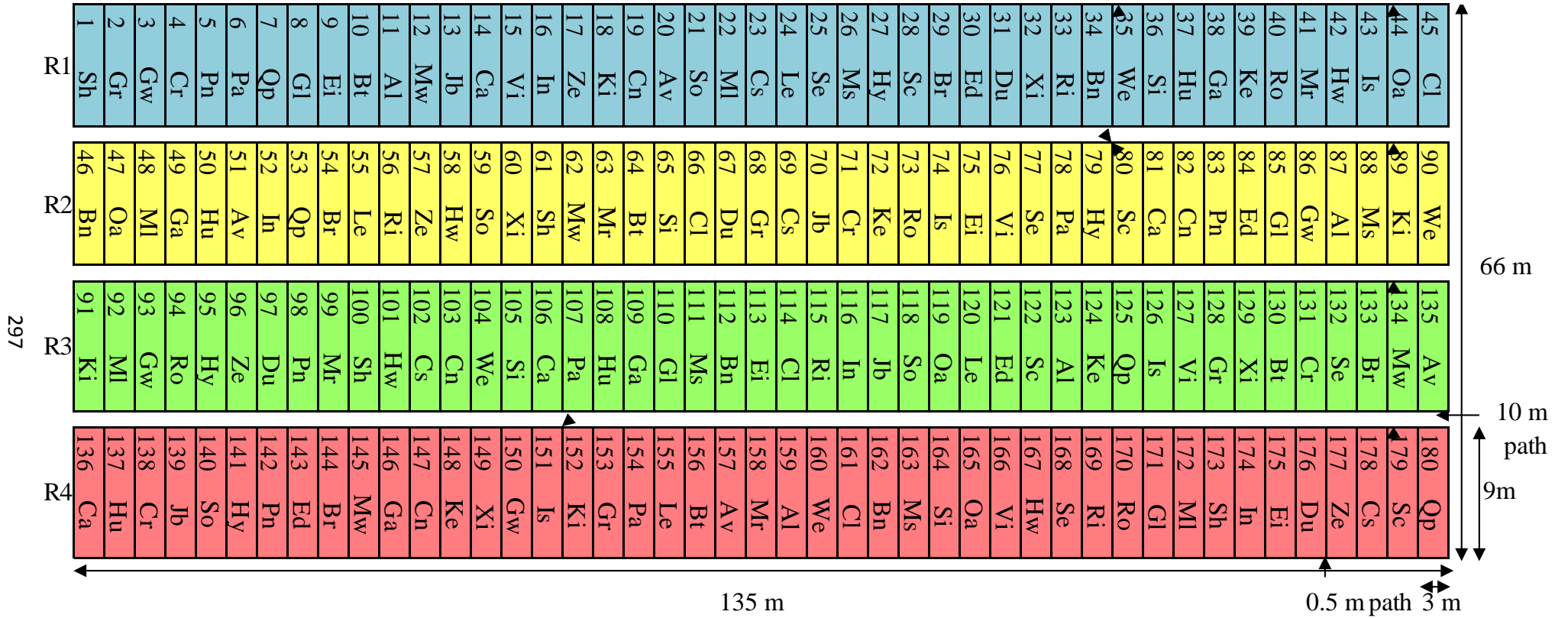


Appendix 6.1. Elite winter wheat varieties and resistance to take-all field plans 2009-2011 (Continued)

Field trial code: 2010/R/WW/1031

Field: West Barnfield I&II

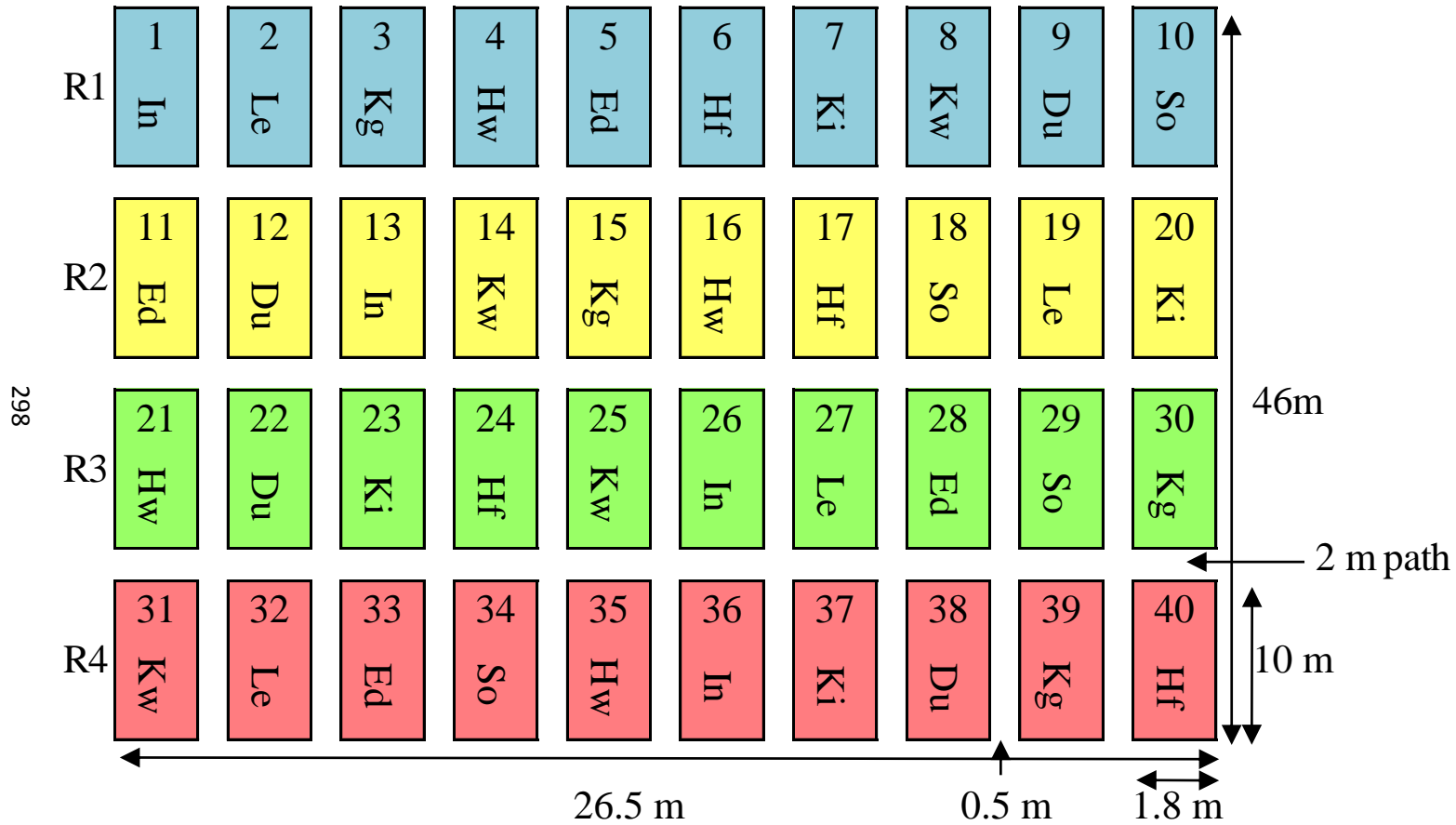
Treatments: 45 winter wheat varieties x 4 reps



Appendix 6.1. Elite winter wheat varieties and resistance to take-all field plans 2009-2011 (Continued)

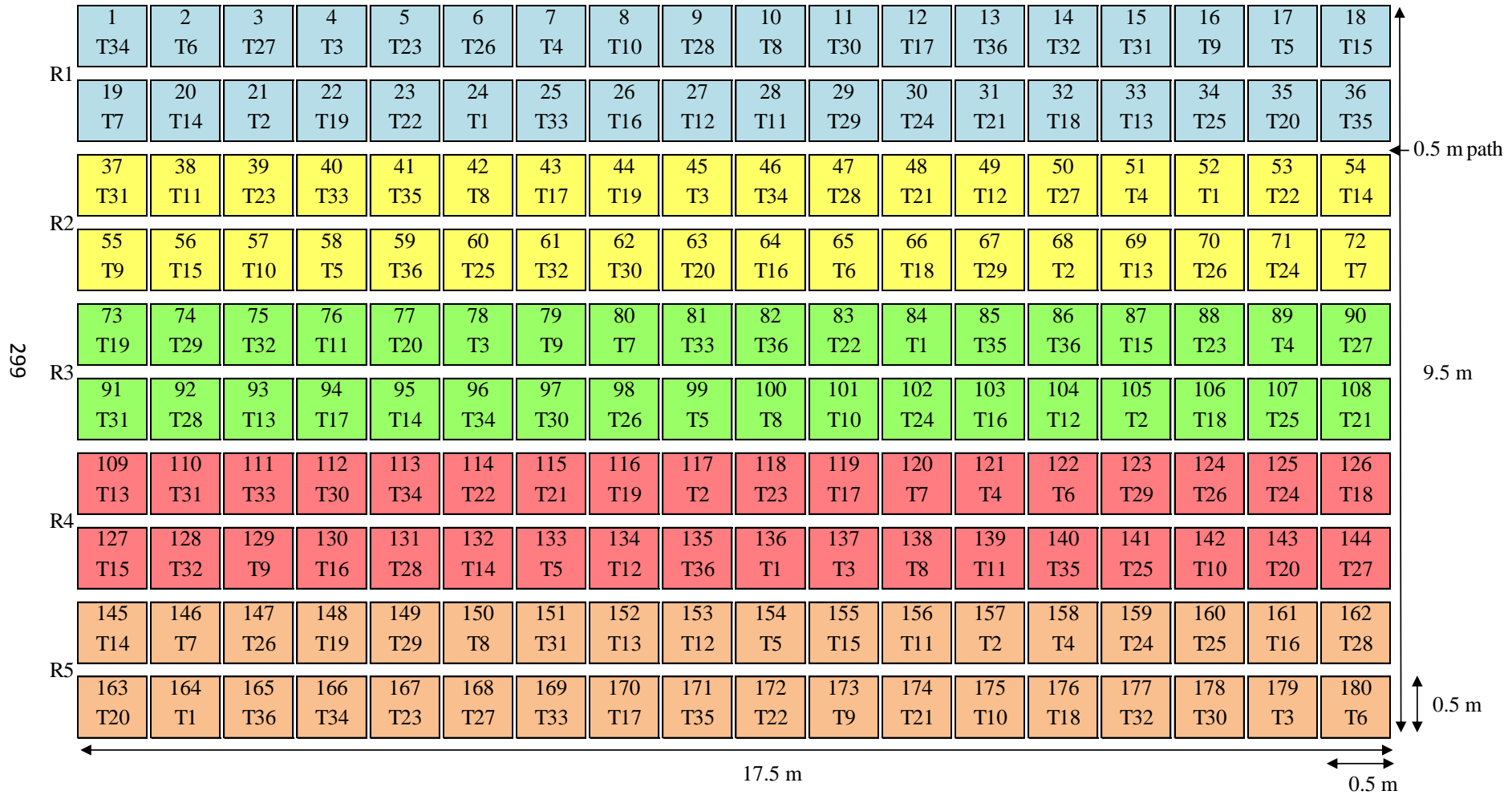
Field trial code: 2011/R/WW/1114 Field: Claycroft

Treatments: 10 winter wheat varieties x 4 reps



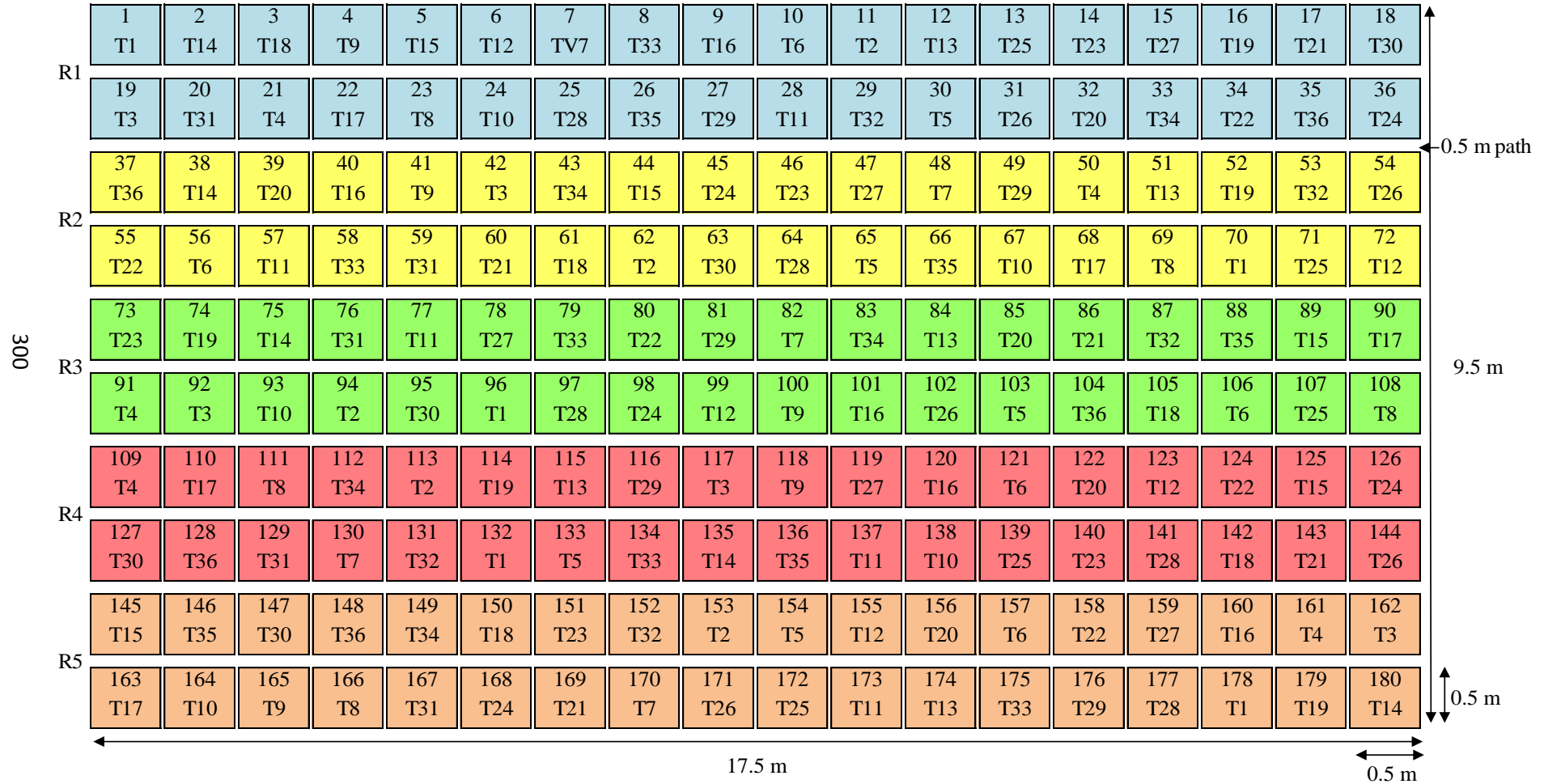
Appendix 7.1. Diploid wheat and resistance to take-all field plans 2006 and 2008-2011

Field trial code: 2006/R/WW/615 Field: Delafield Treatments: 36 x 5 reps (27 *T. monococcum*, 9 hexaploids)



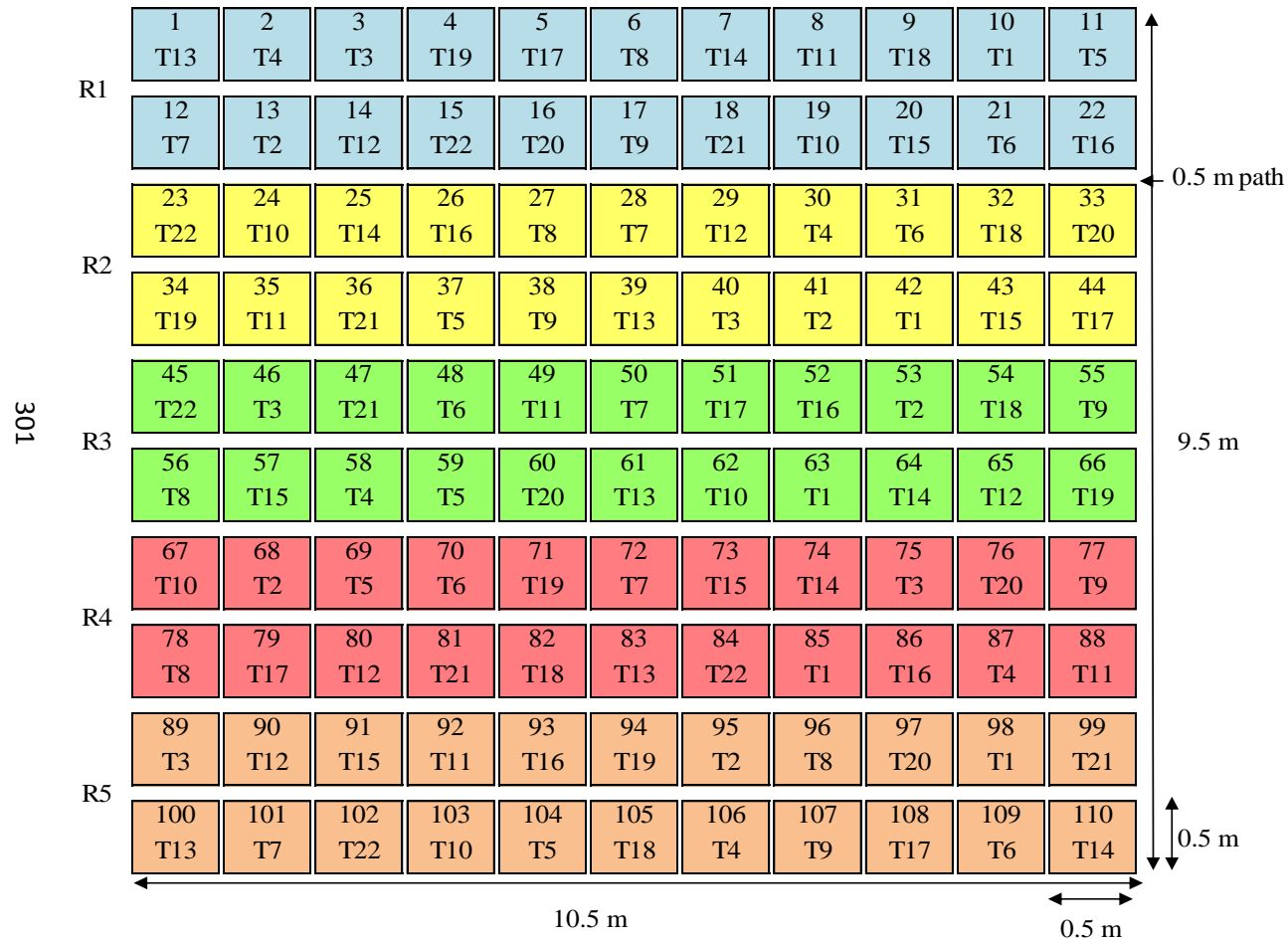
Appendix 7.1. Diploid wheat and resistance to take-all field plans 2006 and 2008-2011 (Continued)

Field trial code: 2008/R/WW/810 Field: Long Hoos I & II Treatments: 36 x 5 reps (19 *T. monococcum*, 3 control species, 14 hexaploids)



Appendix 7.1. Diploid wheat and resistance to take-all field plans 2006 and 2008-2011 (Continued)

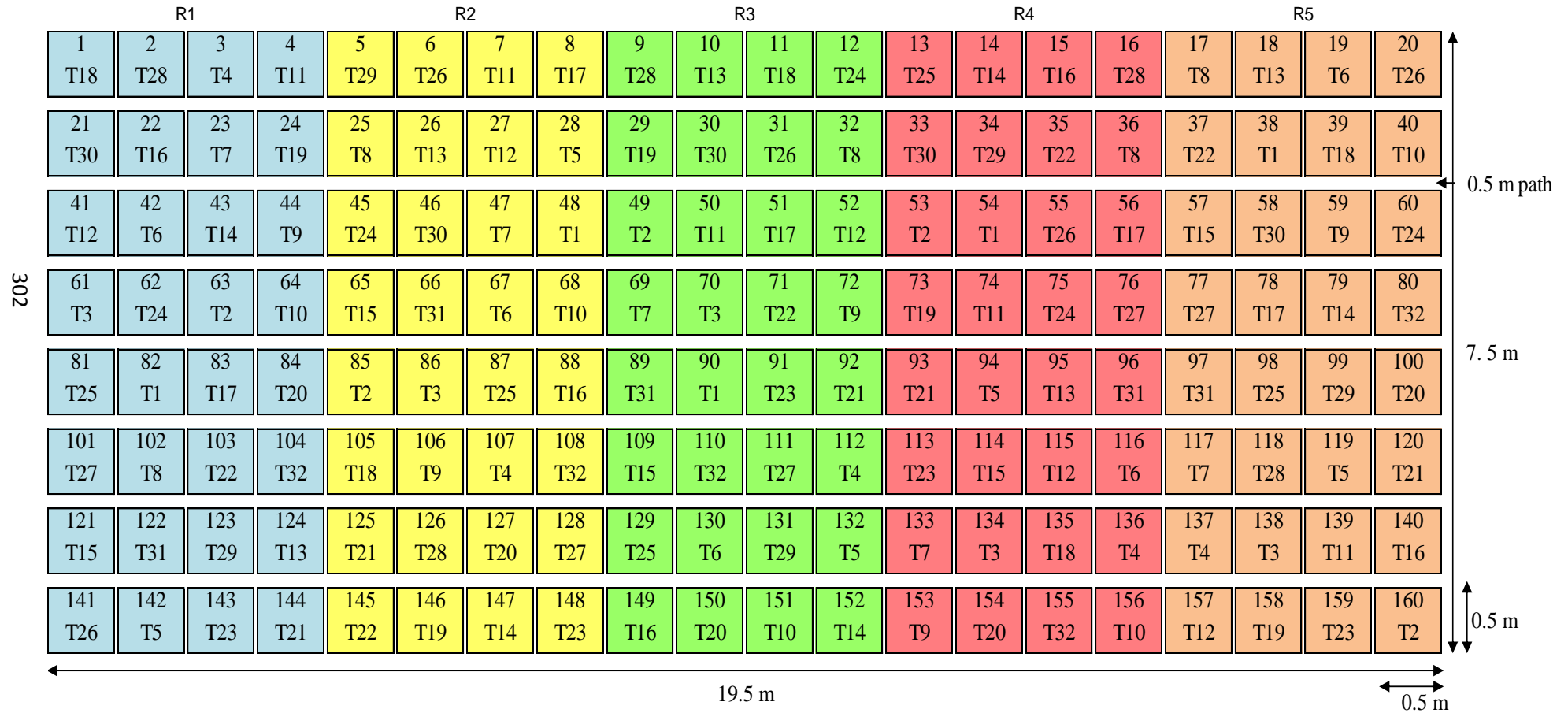
Field trial code: 2009/R/WW/911 Field: Stackyard Treatments: 22 x 5 reps (5 *T. monococcum*, 5 tetraploids, 2 control species, 10 hexaploids)

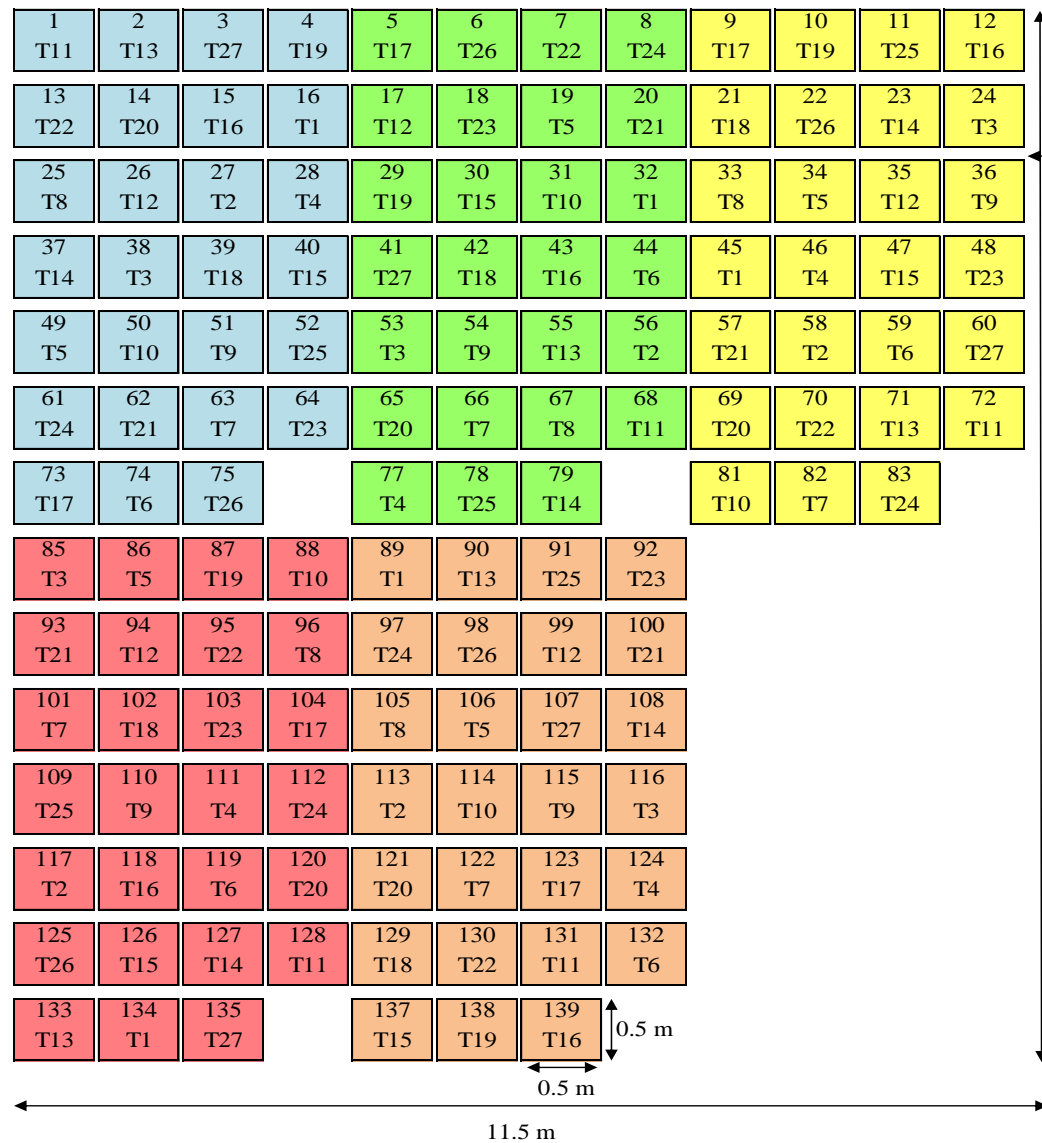


Appendix 7.1. Diploid wheat and resistance to take-all field plans 2006 and 2008-2011 (Continued)

Field trial code: 2010/R/WW/1034 Field: West Barnfield

Treatments: 32 x 5 reps (13 *T. monococcum*, 5 tetraploids, 3 control species, 11 hexaploids, 1 *Aegilops speltoides* accession)





Appendix 7.1. Diploid wheat and resistance to take-all field plans 2006 and 2008-2011 (Continued)

Field trial code: 2011/R/WW/1109

Field: Claycroft

Treatments: 27 x 5 reps (12 *T. monococcum*, 2 control species, 13 hexaploids)

Abbreviations

2,4-DAPG	2,4-diacetylphloroglucinol
AFLP	Amplified fragment length polymorphism
AMF	Arbuscular mycorrhizal fungi
d.f.	Degrees of freedom
DIBOA	2,4-dihydroxy-1,4-benzoxazin-3-one
DIMBOA	2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one
e-RA	electronic Rothamsted Archive
EST	Expressed sequence tag
GFP	Green fluorescent protein
<i>Gga</i>	<i>Gaeumannomyces graminis</i> var. <i>avenae</i>
<i>Ggt</i>	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>
GS	Growth stage
IRD	Infra-red dye
MAB	Marker assisted backcrossing
MAS	Marker assisted selection
Mn	Manganese
Nabim	National Association of British and Irish Millers
NGS	Next generation sequencing
NIL	Near isogenic line
NL	National List
NVZ	Nitrate vulnerable zone
PDA	Potato dextrose agar
qPCR	Quantitative polymerase chain reaction
QTL	Quantitative trait loci
RAPD	Random amplification of polymorphic DNA
RFLP	Restriction fragment length polymorphism
RH	Relative humidity
RL	Recommended List
SDW	Sterile distilled water
SED	Standard error of the difference
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeat
TAB	Take-all inoculum build-up
TAD	Take-all decline
TAI	Take-all index
WGIN	Wheat Genetic Improvement Network

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