ELUCIDATING CROP LOSSES AND CONTROL OF *RHIZOCTONIA SOLANI* AND *RHIZOCTONIA CEREALIS* IN WINTER WHEAT

MATTHEW J. BROWN, BSc (Hons), MSc.

Thesis submitted to the University of Nottingham
for the degree of Doctor of Philosophy

The University of Nottingham
School of Biosciences
Division of Plant and Crop Science
Sutton Bonington Campus
Loughborough, LE12 5RD, UK

FEBRUARY 2016

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my supervisors Dr Rumiana Ray, Dr James Woodhall and Professor Sacha Mooney for their help, support and guidance throughout this study.

Special thanks to Fiona Wilkinson for her support, patience and guidance in the laborator and to Jim Craigon for the statistical advice. Thank you to Matt Tovey and John Alcock for their help and management of the field experiments and to the Food and Environment research agency (Fera) for the use of the laboratories. I would like to specifically thank Kate Perkins and Jackie Wilsenham for their help while I worked at Fera.

I would like to thank Maud Liegeois, Daniel Tomlinson, Linda Nielsen, Esra Özdemir, Abigail Hooley, Marielle Machado and Pierre Schektman for there their help in processing wheat samples and the *Oculimacula* spp. and *Microdochium* spp. molecular work in chapter 3 and 4.

Thank you to AHDB-HGCA for funding Abigail Mason and Daniel Tomlinson, via project RD-2010-3729 'Development of novel methods for detecting and quantifying viable inoculum of *Oculimacula yallundae* and *O. acuformis'* for their work that contributed to *Oculimacula* spp. data used in Chapters 3 and 4.

A special thank you goes to my parents for their unwavering support that without completing this project would not have been possible.

However, the biggest thanks go to all the friends I made during my time in Nottingham; Sarah, Jassy & Sabri, Steve J, Rebecca, Leanne, Steven & Vics, Ruth, Fryni, 'Baby' Daniel, Linda, Masoud, The Beet Group, Abi, Arifa and many more… It was worth it just to meet you.

Finally, I would like to thank the Syngenta for generously funding this PhD project, specifically Christian Schlatter, Monika Joss, Gina Swart, Anja Schade-schuetze and Michael Tait.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS i

LIST OF TABLES xi

LIST OF FIGURES xx

LIST OF ABBREVIATIONS xxiv

CONFERENCE PROCEEDINGS AND PUBLICATIONS xxvi

ABSTRACT xxvii

Chapter 1 1

1.1 INTRODUCTION 2

1.2 *RHIZOCTONIA* 3

1.3 Biology of *Rhizoctonia* spp. 4

1.3.1 Classification 4

1.3.2 Lifecycle of *Rhizoctonia* 5

1.3.2 Hyphal interactions in *Rhizoctonia* 6

1.3.3 Anastomosis groups of binucleate and multinucleate *Rhizoctonia* spp. 7

1.4 Detection and identification of *Rhizoctonia* spp. and anastomosis groups 10

1.4.1 DNA sequencing 10

1.4.2 Nucleic acid amplification 10

1.4.2.1 Conventional PCR 10

1.4.2.2 Real-time PCR 10

1.4.3 Detection in soil 11

1.4.3.1 Plating onto selective media 11

1.4.3.2 Baiting 12

1.4.3.3 Enzyme-linked immunosorbent assay (ELISA) 12

1.4.3.4 Soil DNA extractions and PCR 13

1.5 Rhizoctonia diseases in wheat 14
1.5.1 Damping-off and root rot caused by *R. solani* 14
1.5.2 Sharp eyespot caused by *R. cerealis* 15
1.5.3 Yield losses due to rhizoctonia diseases in wheat 16
1.6 Disease cycle of *R. solani* and *R. cerealis* 17
1.7 Pathogenicity of *Rhizoctonia* spp. to wheat 22
1.7.1 *R. solani* AG 2-1 22
1.7.2 *R. solani* AG 2-2 22
1.7.3 *R. solani* AG 3 22
1.7.4 *R. solani* AG 4 23
1.7.5 *R. solani* AG 5 23
1.7.6 *R. solani* AG 8 23
1.7.7 *R. solani* AG 11 24
1.7.8 Binucleate *Rhizoctonia* spp. 25
1.7.9 *Rhizoctonia* spp. in UK wheat fields 25
1.8 Control of rhizoctonia diseases in wheat 26
1.8.1 Cultural control 26
1.8.2 Biological control 28
1.8.3 Bio-fumigants 29
1.8.4 Host resistance 30
1.8.5 Chemical control 30
1.8.5.1 Seed treatments 31
1.8.5.2 Foliar treatments 32
1.9 Aims and objectives 33
Chapter 2 34
2.0 GENERAL METHODS 35
2.1 Storage and preservation of isolates of *Rhizoctonia* spp. 35

2.1.1 PDA slopes 35
2.1.2 Barley grain 35
2.2 DNA extractions 36
2.2.1 Soil 36
2.2.2 Plant 37
2.2.2.1 CTAB 37
2.2.3 Fungi 38
2.2.3.1 *Rhizoctonia* spp. 38
2.2.3.2 *Oculimacula* spp. and *Microdochium* spp. 38
2.2.4 DNA quantification 39
2.3 Real-time PCR 39
2.3.1 Quantification of *Rhizoctonia spp.* 39
2.3.2 Analysis of real-time PCR (TaqMan®) assays 40
2.3.3 Quantification of *Oculimacula* spp. and *Microdochium* spp. 40
2.4 Visual disease assessments 42
2.4.1 Root rot 42
2.4.2 Eyespot, sharp eyespot and brown foot rot 42
Chapter 3 43
3.1 INTRODUCTION 44
3.2 MATERIALS AND METHODS 47
3.2.1 Soil and plant sampling 47
3.2.2 Disease assessments 48
3.2.3 DNA extractions 48
3.2.3.1 Soil 48
3.2.3.2 Plant 48
3.2.4 Real-time PCR assays 48
3.2.5 Meteorological data 49

3.2.6 Statistical analysis 50
3.3 RESULTS 51
3.3.1 Incidence and severity of root rot and stem base diseases 51
3.3.2 Pathogen presence and DNA concentrations in soil 53
3.3.3 Pathogen presence and DNA concentrations in roots 56
3.3.4 Pathogen presence and DNA concentrations in stem bases 58
3.3.5 Pathogen presence and DNA concentrations in stems 60
3.3.6 Meteorological data for English wheat fields sampled in 2011/12 and 2012/13 61
3.3.7 Correlation coefficients (r) between root rot and SBDs with meteorological variables 62
3.3.8 Correlation coefficients (r) between pathogen DNA in soil and *planta* correlated with and meteorological variables 64
3.3.8.1 Soil 64
3.3.8.2 Roots 64
3.3.8.3 Stem bases 65
3.3.8.4 Stems 65
3.3.9 Principal component analysis (PCA) for diseases, pathogen DNA and meteorological variables in soil, roots, stem bases and stems 70
3.3.9.1 GS 21-31 70
3.3.9.2 GS 37-45 73
3.3.9.3 GS 65-75 76
3.4 DISCUSSION 79
3.4.1 Disease occurrence in English wheat crops 79
3.4.2 Population dynamics of pathogens in field soil 81
3.4.3 Population dynamics of pathogens in root tissue 84
3.4.4 Population dynamics of pathogens in stem tissue 85
3.5 CONCLUSIONS 88

Chapter 4 90
4.1 INTRODUCTION 91
4.2 MATERIALS AND METHODS 95
4.2.1 Soil and plant sampling 95
4.2.2 Visual disease assessments 95
4.2.3 Soil and plant DNA extractions 95
4.2.4 Agronomy factors 95
4.2.5 Meteorological data 95
4.2.6 Statistical analysis 96
4.3 RESULTS 100
4.3.1 Agronomy and meteorological factors influencing disease index (DI) of root rot and stem bases diseases and pathogen DNA during crop development 100
4.3.1.1 Disease index 100
4.3.1.2 Pathogen DNA in soil 100
4.3.1.3 Roots 101
4.3.1.4 Stem bases 101
4.3.2 Development of root rot and stem base diseases 107
4.3.3 Seasonal effects on pathogen DNA accumulation in soil, roots and stem bases 109
4.3.4 Influence of agronomy factors on individual diseases and pathogens throughout crop development 113
4.3.4.1 Soil texture 113
4.3.4.2 Previous crop 113
4.3.4.3 Position of wheat in the rotation 117
4.3.4.4 Sowing date 118
4.3.4.5 Eyespot resistance rating of the growing crop 121
4.3.4.6 Seed treatment 121

4.3.5 Influence of environmental factors on individual disease and pathogens throughout crop development 124
4.3.5.1 Temperature 124
4.3.5.2 Total rainfall 125
4.3.5.3 Relative humidity 126
4.3.6 Effect of fungicides applied at GS 31-32 (T1) and GS39 (T2) on pathogen DNA and stem base diseases at GS 37-45 and GS 65-75 131
4.4 DISCUSSION 132
4.5 CONCLUSIONS 139
Chapter 5 140
5.2 INTRODUCTION 141
5.2 Materials and methods 146
5.2.1 Experimental design 146
5.2.2 Inoculum production 147
5.2.3 Agronomy 147
5.2.4 Plant sampling and crop assessments 148
5.2.5 Seedling emergence and assessment of plant populations 149
5.2.6 Visual disease assessments 149
5.2.7 Soil sampling and pathogen DNA extractions 149
5.2.8 Pathogen DNA extraction from plant material 149
5.2.9 Pathogen quantification using real-time PCR 150
5.2.10 Green area index, dry weight and ear partitioning index 150
5.2.11 Spectral reflectance assessments 152
5.2.12 Yield components 153
5.2.13 Statistical analysis 153
5.3 RESULTS 154
5.3.1 Emergence and plant populations 154
5.3.2 Root rot disease and effect of seed treatments 157

5.3.3 Stem base diseases and effects of seed treatments 162

5.3.3.1 Stem browning 162

5.3.3.2 Stem base diseases 162

5.3.4 Pathogen DNA in soil at GS 15 172

5.3.5 Effect of inoculation and seed treatment *on Rhizoctonia* DNA in roots…...……...172

5.3.6 Effect of inoculation and seed treatment *on Rhizoctonia* DNA in stems…..……...172

5.3.7 Effect of inoculation and seed treatment *on Microdochium* spp. DNA in stems ……...173

5.3.8 Physiological assessments..... 182

5.3.9 Yield components 185

5.3.9.1 Plant height (mm) (GS 15) 185

5.3.9.2 Canopy green area (cm^2) (GS 15) 185

5.3.9.3 Above ground dry matter (AGDM) ($g\ m^2$) 185

5.3.9.4 Green area index (GAI) 185

5.3.9.5 Grain yield components..... 186

5.3.10 Relationships between disease indexes and pathogen DNA 190

5.3.11 Multiple linear regression of yield components with plant developmental assessments, root and stem disease indexes and spectral reflectance indices..... 190

5.3.12 Meteorological data for 2012/13 and 2013/14 field experiments ... 195

5.4 DISCUSSION 196

5.4.1 Plant emergence, root rot and stem base diseases and their pathogens…….196

5.4.2 Effects of seed treatment on root rot and stem base diseases and their pathogens diseases and pathogens 198

5.4.3 Spectral reflectance measurements..... 201

5.4.4 Yield components 202

5.5 CONCLUSIONS 203
Chapter 6 205
6.1 INTRODUCTION 206
6.2 rDNA-ITS sequencing of *Rhizoctonia* spp. 207
6.2.1 Introduction 207
6.2.2 Materials and methods 209
6.2.3 ITS sequencing of *Rhizoctonia* spp. 209
6.2.4 Origin and collection of isolates 209
6.2.5 DNA extraction 210
6.2.6 PCR 210
6.2.7 DNA sequencing 210
6.2.8 Results 211
6.2.9 Anastomosis groups 211
6.2.10 Analysis of rDNA ITS sequence diversity in *Rhizoctonia* spp. 211
6.2.11 Discussion 216
6.2.12 Anastomosis groups 216
6.2.13 rDNA ITS sequencing 216
6.3. Aggressiveness of *Rhizoctonia* spp. to wheat 218
6.3.1 Introduction 218
6.3.2 Materials and methods 220
6.3.3 Isolates and experimental design 220
6.3.4 Plant growth measurements and visual disease assessments 221
6.3.5 Statistical analysis 221
6.3.6 Results 222
6.3.7 Discussion 232
6.4 Sensitivity of *Rhizoctonia* spp. to fungicides 235
6.4.1 Introduction 235

6.4.2 Materials and methods 236
6.4.3 Isolates and fungicides 236
6.4.4 Sighting experiment 236
6.4.5 Sensitivity of *Rhizoctonia* spp. to fludioxonil, prothioconazole and sedaxane 236
6.4.6 Statistical analysis 237
6.4.7 Results 239
6.4.8 Discussion 243
6.5 Conclusions 245
Chapter 7 247
7.1 GENERAL DISCUSSION 248
7.2 FUTURE WORK 254
8. REFFERENCES 256
APPENDIX - A 290
APPENDIX 1 299
Appendix 2 302
Appendix 3 303
Appendix 4 304
Appendix 5 306
Appendix 6 308
Appendix 7 310
Appendix 8 311
Appendix 9 312
Appendix 10 313

LIST OF TABLES

Table 1.1 Previous categorisation systems for anastomosis reactions between *Rhizoctonia* isolates (adapted from Carling, 1996) 7

Table 1.2 Summary of the different groups and subgroups recognized for multinucleate *Rhizoctonia solani* and their hosts 8

Table 1.3 Summary of the different groups and subgroups recognized for binucleate *Rhizoctonia* spp. and their hosts 9

Table 2.1. Soil sample amount and related materials for DNA extractions 37

Table 3.1 Pathogens targeted in soil, root, stem bases or stems by real-time PCR assays 49

Table 3.2 Mean (%) disease incidence (I) and disease index (DI) for root rot, eyespot, brown foot rot, sharp eyespot on English winter wheat plants collected at GS21-31, GS 37-45 and GS65-75 in 2011/12 and 2012/13 52

Table 3.3 Pathogen DNA in soil samples from English winter wheat fields collected at pre-sowing , GS 21-31, GS 37-45 and GS 65-75 in 2011/12 and 2012/13 seasons 55

Table 3.4 Pathogen DNA in roots of English winter wheat plants collected at GS21-31, GS 37-45 and GS65-75 in 2011/12 and 2012/13 57

Table 3.5 Pathogen DNA in stem bases (5 cm stem basal region) of English winter wheat plants collected at GS21-31, GS37-45 and GS 65-75 in 2011/12 and 2012/13 59

Table 3.6 Pathogen DNA in stems (15 cm above the 5 cm basal region) of English winter wheat plants collected at GS37-45 and GS 65-75 in 2011/12 and 2012/13 60

Table 3.7 Mean temperature, relative humidity and total rainfall for fields sampled from pre-sowing to GS 65-75 across England 61

Table 3.8 Correlation coefficients (*r*) and probabilities (in parentheses) if $P \leq 0.05$ between disease index on roots and stems assessed at GS21-31, GS37-45 and GS65-75 in 2011/12 to 2012/13 and temperature (*max* and *min*), total rainfall and relative humidity in English winter wheat fields 63

Table 3.9 Correlation coefficients (*r*) and probabilities (in parentheses) between pathogen DNA concentrations (log pg g^{-1} of soil) in soil samples collected at GS21-31, GS37-45 and GS65-75 in 2011/12 to 2012/13 temperature (*max* and *min*), total rainfall and relative humidity in English winter wheat fields 66

Table 3.10 Correlation coefficients (*r*) and probabilities (in parentheses) between pathogen DNA concentrations (log pg ng^{-1} of total DNA) in roots collected at GS21-31, GS37-45 and GS65-75 in 2011/12 67

Table 3.11 Correlation coefficients (*r*) and probabilities (in parentheses) between pathogen DNA concentrations (log pg ng^{-1} of total DNA) in stem bases (5 cm stem basal region) collected at GS21-31, GS37-45 and GS65-75 in 201112 to 2012/13 and temperature (*max* and *min*), total rainfall and relative humidity in English winter wheat fields 68

Table 3.12 Correlation coefficients (*r*) and probabilities (in parentheses) between pathogen DNA concentrations (log pg ng^{-1} of total DNA) in stems (15 cm above the 5 cm basal region) collected at GS37-45 and GS65-75 in 201112 to 2012/ and temperature (*max* and *min*), total rainfall and relative humidity in English winter wheat fields 69

Table 4.1 Agronomical information gathered for English wheat fields sampled in 2011/12 and 2012/13. In parenthesis [-] number of observations for each factor 97

Table 4.2 Active ingredients applied as seed treatments or T1 (GS31-32) and T2 (GS39) foliar fungicides to English wheat crops sampled in 2011/12 and 2012/13. In parenthesis [-] number of observations for each factor..... 98

Table 4.3 Temperature (*minima* and *maxima*) (°C), total rainfall (mm) and relative humidity (%) for the locations of English wheat crops sampled in 2011/12 and 2012/13. In parenthesis [-] number of observations for each factor. 99

Table 4.4 Agronomy and meteorological factors effecting the DI of root rot, sharp eyespot, eyespot and BFR in English wheat crops in 2011/12 and 2012/13 during crop development (sampled at time GS21-31, GS37-45 and GS65-75) analysed by residual maximum likelihood (REML) analysis ($P \leq 0.05$). 103

Table 4.5 Agronomy and meteorological factors effecting pathogen DNA (pg g^{-1} of soil) in soil samples collected from English wheat fields in 2011/12 and 2012/13 at GS21-31, GS37-45 and GS65-75 analysed by residual maximum likelihood (REML) analysis (P≤0.05). 104

Table 4.6 Agronomy and meteorological factors effecting pathogen DNA (pg ng^{-1} of total DNA) in roots of wheat plants collected from English wheat fields in 2011/12 and 2012/13 at GS21-31, GS37-45 and GS65-75 analysed by residual maximum likelihood (REML) analysis (P≤0.05). 105

Table 4.7 Agronomy and meteorological factors effecting pathogen DNA (pg ng^{-1} of total DNA) in stem bases of wheat plants collected from English wheat fields in 2011/12 and 2012/13 at GS21-31, GS37-45 and GS65-75 analysed by residual maximum likelihood (REML) analysis (P≤0.05). 106

Table 4.8 Effect of season (2011/12 and 2012/13) on root and stem diseases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 108

Table 4.9 Effect of season (2011/12 and 2012/13) on pathogen DNA in soil and roots. Values are REML-predicted means from wheat crops sampled T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 111

Table 4.10 Effect of season (2011/12 and 2012/13) on pathogen DNA in stem bases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 112

Table 4.11 Effect of soil texture on pathogen DNA in soil and roots and disease. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 115

Table 4.12 Effect of previous crop on pathogen DNA in soil and stem bases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 116

Table 4.13 Effect of position of wheat in the rotation (1st, 2nd or continuous wheat (CW)) on pathogen DNA in roots and stem bases and diseases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 119

Table 4.14 Effect of sowing date on pathogen DNA in soil, roots and stem bases and diseases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 120

Table 4.15 Effect of eyespot resistance rating (ER) on pathogen DNA in roots and stem bases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 122

Table 4.16 Effect of seed treatments on pathogen DNA in roots. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 123

Table 4.17 Effect of mean $Temp_{max}$ (^{o}C) over the autumn/winter period on pathogen DNA in soil and stem bases and disease. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 127

Table 4.18 Effect of mean $Temp_{min}$ (^{o}C) over the autumn/winter period on pathogen DNA in soil and stem bases and diseases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 128

Table 4.19 Effect of total rainfall (mm) over the autumn/winter period on pathogen DNA in soil and disease. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 129

Table 4.20 Effect of mean relative humidity (%) over the autumn/winter period on pathogen DNA in stem bases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-). 130

Table 5.1 Inoculation and seed treatment combinations for 2012/13 and 2013/14 field experiments 147

Table 5.2 Spectral reflectance indices (SRI) for wheat canopy analysis at GS39, GS75 and GS85 for 2012/13 and 2013/14 field experiments 152

Table 5.3 Emergence (%) of wheat seedlings 4 weeks after sowing. Seed was treated with sedaxane and/or fludioxonil or untreated and sown into field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis* 155

Table 5.4 Plant population (m^2) at GS 39 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis* 156

Table 5.5 Root rot disease index (%) at GS 15 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis) 158

Table 5.6 Root rot disease index (%) at GS 31 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis) 159

Table 5.7 Root rot disease index (%) at GS 39 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis) 160

Table 5.8 Root rot disease index (%) at GS 75 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (back-transformed means in parenthesis) 161
Table 5.9 Stem browning disease index (%) at GS 15 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis) 164
Table 5.10 Sharp eyespot disease index (%) at GS 31 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis) 166
Table 5.11 Sharp eyespot disease index (%) at GS 39 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis) 167
Table 5.12 Sharp eyespot disease index (%) at GS 75 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis). 168
Table 5.13 Brown foot rot index (%) at GS 31 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis* in 2012/13 and 2013/14 experiments. Angular transformed disease index (%) (non-transformed means in parenthesis). 169
Table 5.14 Brown foot rot index (%) at GS 39 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis* in 2012/13 and 2013/14 experiments. Angular transformed disease index (%) (non-transformed means in parenthesis). 170

Table 5.15 Brown foot rot index (%) at GS 75 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis* in 2012/13 and 2013/14 experiments. Angular transformed disease index (%) (non-transformed means in parenthesis). 171

Table 5.16 *Rhizoctonia* DNA (pg g^{-1} of soil) in soil collected at GS 15 of wheat plants grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis 174

Table 5.17 *Rhizoctonia* DNA (pg ng^{-1} of total DNA) in wheat roots at GS 31 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis 175

Table 5.18 *Rhizoctonia* DNA (pg ng^{-1} of total DNA) in wheat roots at GS 75 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis 176

Table 5.19 *Rhizoctonia* DNA (pg ng^{-1} of total DNA) in wheat stems at GS 15 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis 177

Table 5.20 *Rhizoctonia* DNA (pg ng^{-1} of total DNA) in wheat stems at GS 31 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis 178

Table 5.21 *Rhizoctonia* DNA (pg ng^{-1} of total DNA) in wheat stems at GS 75 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis 179

Table 5.22 *M. nivale* DNA (pg ng^{-1} of total DNA) in wheat stems at GS 15 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis. M. nivale* DNA values are log_{10} transformed with back-transformed means in parenthesis 180

Table 5.23 *M. majus* DNA (pg ng^{-1} of total DNA) in wheat stems at GS 15 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis. M. majus* DNA values are log_{10} transformed with back-transformed means in parenthesis 181

Table 5.24 Plant height (mm) of wheat at GS 15 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis* 187

Table 5.25 Green area (cm^2) of wheat at GS 15 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1 and AG 5 and *R. cerealis* 188

Table 5.26 Harvest yield components for ear portioning index, grain yield (t ha^{-1}), specific weight (SPW), thousand grain weight (TGW) for wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. 189

Table 5.27 Summary of significant regressions for stem base diseases and log_{10} DNA of *Rhizoctonia* spp. in stems for 2012/13 and 2013/14 field experiments 192

Table 5.28 Summary of significant regressions for yield (t ha^{-1}), root and stem diseases and log_{10} DNA of *Rhizoctonia* spp. for the 2012/13 and 2013/14 field experiments 193

Table 5.29 Multiple linear regression models of the 2012/13 and 2013/14 field experiments accounting for variation in yield components using plant developmental assessments, root and stem disease indexes and spectral reflectance indices at GS 15, GS 31, GS 39, GS 75 and GS 85. 194

Table 5.30 Mean temperature, total rainfall and relative humidity from sowing to GS 75 in 2012/13 and 2013/14 field experiments. 195
Table 6.1 Distribution (field and county) of the 160 *R. cerealis* isolates cultured from English wheat crops along with ITS type sequenced and presence (✓) or absence (×) of *R. cerealis* DNA 214
Table 6.2 Fungicide name, class, mode of action and chemical structure..... 238
Table 6.3. EC_{50} values of prothioconazole, fludioxonil and sedaxane for a range of *R. cerealis* isolates (n = 25) cultured from wheat plants in 2012 and 2014. . 240
Table 6.4. EC_{50} values of prothioconazole, fludioxonil and sedaxane for a range of anastomosis groups of *R. solani* isolates..... 241

LIST OF FIGURES

Figure 1.1 Rhizoctonia root rot on wheat roots 14
Figure 1.2 Sharp eyespot symptoms on wheat stems 15
Figure 1.3 Disease cycle of rhizoctonia root rot on wheat 18
Figure 1.4 Disease cycle of sharp eyespot caused by *R. cerealis*..... 18
Figure 3.1 Biplot of the principle component analysis showing relationships between pathogens, disease and meteorological variables in a) soil, b) roots and c) stem bases of winter wheat at GS 21-31. *M. nivale* (Mn), *M. majus* (Mm), *O. acuformis* (OA), *O. yallundae* (OY), *R. cerealis* (Rc) , *R. Solani* AG 2-1 (AG 2-1), eyespot, sharp eyespot (S. eyespot) and brown foot rot (BFR) and disease index (DI). Total rainfall (TR), relative humidity (RH) and mean daily maximum (T-max) and minimum temperature (T-min). (*Continued*) 71
Figure 3.2 Biplot of the principle component analysis showing associations between pathogens, disease and meteorological variables in a) soil, b) roots and c) stem bases and d) stems of winter wheat at GS 37-45. *M. nivale* (Mn), *M. majus* (Mm), *O. acuformis* (OA), *O. yallundae* (OY), *R. cerealis* (Rc) , *R. Solani* AG 2-1 (AG 2-1), eyespot, sharp eyespot (S. eyespot) and brown foot rot (BFR) and disease index (DI). Total rainfall (TR), relative humidity (RH) and mean daily maximum (T-max) and minimum temperature (T-min). (*continued*) 74
Figure 3.3 Biplot of the principle component analysis showing associations between pathogens, disease and meteorological variables in a) soil, b) roots and c) stem bases and d) stems of winter wheat crops at GS 65-75. *M. nivale* (Mn), *M. majus* (Mm), *O. acuformis* (OA), *O. yallundae* (OY), *R. cerealis* (Rc) , *R. Solani* AG 2-1 (AG 2-1), eyespot, sharp eyespot (S. eyespot) and brown foot rot (BFR) and disease index (DI). Total rainfall (TR), relative humidity (RH) and mean daily maximum (T-max) and minimum temperature (T-min).(*Continued*) 77
Figure 4.1 The effect of T1 fungicides on brown foot rot (BFR) at GS 37-45. MSI - multi-site inhibitors, Prothio – prothioconazole, Strob – strobilurin. Numbers above bars are number of observations. SED – Average for comparing all means. SED – Min for comparing means for the two highest number of observations, SED – Max for comparing the means of the two lowest number of observations. 131

Figure 5.1 Wintersteiger plot drill used in the 2012/13 field experiment 148
Figure 5.2 Effect of seed treatment on stem browning of wheat grown in *R. cerealis* inoculated plots 165
Figure 5.3 Effect of seed treatment on stem browning of wheat grown in AG 5 inoculated plots..... 165
Figure 5.4 Photochemical reflectance index (PRI) for wheat plants at GS 39, GS 75 and GS 85 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis.* 183
Figure 5.5 Photochemical reflectance index (PRI) for wheat plants at GS 39, GS 75 and GS 85 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots. 183
Figure 5.6 Normalized water index 2 (NWI-2) for wheat plants at GS 39, GS 75 and GS 85 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots. 184
Figure 5.7 Ratio analysis of reflectance spectra carotenoid (RARSc) for wheat plants at GS 39, GS 75 and GS 85 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots. 184
Figure 6.1. Number of rDNA ITS sequence variants in 160 UK isolates of *R. cerealis* and corresponding nucleotide positions of their polymorphism..... 213
Figure 6.2. Phylogenetic tree based on the rDNA-ITS sequences of UK *R. cerealis* (AG-D) isolates (labelled MB1 to 160) with reference sequences from GenBank. The tree was built using the neighbour joining method. The numbers at branches were the percentage of trees containing the corresponding clade based on 1,000 bootstrap replications. AG-E was used as an outgroup 215
Figure 6.3. Emergence (%) of pre-germinated winter wheat (cv. Santiago) inoculated with *R. cerealis* isolates 10 days post planting conducted over two experiments. 1 = experiment 1 and 2 = experiment 2. Dotted line represents the approximate point where emergence of the inoculated became significantly less ($P < 0.05$) than the emergence for the unon-inoculated control (red line). Bar = least significant difference ($P \leq 0.05$)..... 224

Figure 6.4. Sharp eyespot disease index (DI%) for 10 day old winter wheat (cv. Santiago) seedlings inoculated with *R. cerealis* isolates conducted over two experiments. 1 = experiment 1 and 2 = experiment 2. Dotted line represents the approximate point where sharp eyespot of the inoculated became significantly more (P <0.05) than for the non-inoculated control. Bar = least significant difference (P≤0.05). Data is angular-transformed. 225
Figure 6.5. Plant height (cm) of 10 day old winter wheat (cv. Santiago) seedlings inoculated with *R. cerealis* isolates conducted over two experiments. 1 = experiment 1 and 2 = experiment 2. Dotted line represents the approximate point where plant height of the inoculated became significantly more (P <0.05) than for the non-inoculated control. Bar = least significant difference (P≤0.05). Data is angular-transformed 226
Figure 6.6. Pre-emergence damping off caused by *R. cerealis* isolate 2029 (left) and the non-inoculated control (right) after 10 days growth. 227
Figure 6.7. Stunted development of 10 day old wheat seedlings inoculated with *R. cerealis* isolate 1486 (left) and the non-inoculated control (right). 227
Figure 6.8. Emergence (%) of pre-germinated winter wheat (cv. Santiago) inoculated with *Rhizoctonia* spp. 10 days post planting in experiment 4. Dotted line represents the approximate point where emergence of the inoculated became significantly less (P <0.05) than for the non-inoculated experimental control (red line). Bar = least significant difference (P≤0.05). Data is angular-transformed. 228
Figure 6.9. Root rot disease index (DI%) of 10 day old winter wheat (cv. Santiago) inoculated with *Rhizoctonia* spp. 3 = experiment 3 and 4 = experiment 4. Dotted line represents the approximate point where root rot of the inoculated became significantly less (P <0.05) than the non-inoculated experimental control (red line). Bar = least significant difference (P≤0.05). Data is angular-transformed. 229
Figure 6.10. Rhizoctonia stem browning disease index (DI%) of 10 day old winter wheat (cv.. Santiago) inoculated with *Rhizoctonia* spp. 3 = experiment 3 and 4 = experiment 4. Dotted line represents the approximate point where stem browning of the inoculated became significantly less (P <0.05) than for the non-inoculated experimental control (red line). Bar = least significant difference (P≤0.05). Data is angular-transformed. 230

Figure 6.11. Plant height of 10 day old winter wheat (cv. Santiago) inoculated with *Rhizoctonia* spp. 3 = experiment 3 and 4 = experiment 4. Dotted line represents the approximate point where plant height of the inoculated became significantly (P <0.05) lesser/greater than for the non-inoculated experimental control (red line). Bar = least significant difference (P≤0.05). Data is angular-transformed. 231

Figure 6.12. Growth of AG 4-II (1846) on sedaxane amended plates in seven 10-fold dilutions from 0.5 ppm to 0.008 ppm and a control amended with methanol (MeOH). 242

Figure 6.13. Growth of isolate 1739 on (a) sedaxane and (b) fludioxonil amended plates in seven 10-fold dilutions from 0.5 ppm to 0.008 ppm and a control amended with methanol (MeOH)..... 242

LIST OF ABBREVIATIONS

a.i – Active ingredient

AG – Anastomosis group

AHDB - Agricultural and horticultural developmental board

BFR – Brown foot rot

EC_{50} - Effective concentration causing 50% growth inhibition of fungal mycelium

ECMWF - European Centre for Medium-Range Weather Forecasts

Fera – Food and Environmental Research Agency

GAI – Green area index

G-NDVI - Green normalized difference vegetation index

GPS - Globally Positioning System

GS – Growth stage

h – Hour

Log_{10} - Logarithm base 10

MEC_{50} – Mean effective concentration causing 50% growth inhibition of fungal mycelium

MLR – Multiple linear regression

NDVA - Normalized difference vegetation index

nm – Nanometre

NWI-1 - Normalized water index 1

NWI-2 - Normalized water index 2

NWI-3 - Normalized water index 3

NWI-4 - Normalized water index 4

PCA – Principal component analysis

PDA – Potato dextrose agar

PRI - Photochemical reflectance index

Q-PCR – Real-time polymerase chain reaction

RARSa - Ratio analysis of reflectance spectra chlorophyll a

RARSc - Ratio analysis of reflectance spectra carotenoid

R-NDVI - Red normalized difference vegetation index

SBD – stem base disease

SMB - Sabouraud maltose broth

Sra - Simple ratio

SRM - Spectral reflectance measurements

SW – Specific weight

TGW – Thousand grain weight

WI - Water index

CONFERENCE PROCEEDINGS AND PUBLICATIONS

BROWN, **M.J**., WOODHALL, J., MOONEY, S.J., and RAY, R.V. 2014. The occurrence and population dynamics of *Rhizoctonia solani* in soil of winter wheat. *The Dundee Conference. Crop Protection in Northern Britain 2014, Dundee, UK, 25-26 February 2014*. The Association for Crop Protection in Northern Britain, 2014.

(Appendix – A)

STURROCK, C. J., WOODHALL, J., **BROWN**, **M**., WALKER, C., MOONEY, S. J. & RAY, R. V. 2015. Effects of damping-off caused by *Rhizoctonia solani* anastomosis group 2-1 on roots of wheat and oil seed rape quantified using X-ray Computed Tomography and real-time PCR. *Frontiers in Plant Science,* 6.

Open access journal, paper avaiable from:
http://journal.frontiersin.org/article/10.3389/fpls.2015.00461/full

ABSTRACT

Rhizoctonia solani is a species complex comprising 13 anastomosis groups (AG) that cause disease in a broad range of crops. Various AGs of *R. solani* are pathogenic to winter wheat causing damping-off and root rots. *Rhizoctonia cerealis,* the fungal agent of sharp eyespot, is known to commonly occur on the stems of wheat as part of stem base disease complex (SBD) consisting also of eyespot and brown foot rot (BFR). Eyespot is caused by *Oculimacula acuformis* or *O. yallundae* and BFR is principally caused by *Microdochium nivale* or *M. majus*. The population dynamics of SBD pathogens are continually changing due to environmental and agronomic pressures. Therefore, information on the current population dynamics and factors that influence population changes can guide the implementation of effective control measures. Furthermore the presence and dynamics of *Rhizoctonia* spp. in English wheat crops has not been previously investigated and there is also limited knowledge on the yield losses associated with root and stem base rhizoctonia diseases in wheat.

This work aimed to elucidate crop losses and control of *R. solani* and *R. cerealis* in English wheat crops. An investigation was conducted into the incidence and severity of root and stem base diseases and population dynamics of their associated pathogens in 102 English winter wheat crops. Crop losses and control of the main *Rhizoctonia* spp. identified in English wheat crops were further investigated in artificially inoculated field experiments. Additional characterisation of *Rhizoctonia* spp. was investigated using ITS sequencing, isolate pathogenicity and *in vitro* fungicide sensitivity experiments.

The predominant AG of *R. solani* in English winter wheat crops identified in 63% of soil samples was AG 2-1 with the highest DNA concentrations found in soil where the previous crop was oilseed rape. This suggests that OSR in the rotation is selecting for AG 2-1. Field experiments showed that wheat was not a major host of AG 2-1 since the pathogen failed to cause significant effects on wheat growth and yield.

Sharp eyespot co-occurred with eyespot and BFR in English wheat crops. In 2011/12 at GS 65-75 sharp eyespot symptoms and *R. cerealis* DNA were detected in 90 and 94% of crops, respectively. The population structure of *R.*

cerealis in English wheat crops revealed all isolates belonged to the subgroup AG D-I. Sharp eyespot incidence and severity showed considerable season to season variation primarily due to environmental conditions with cool, wet and humid summer conditions favouring the disease. However, agronomy practices did not have a major influence on sharp eyespot and *R. cerealis* DNA in soil or *in planta*. In field experiments *R. cerealis* caused significant plant establishment losses, stem browning and reduction in grain yield of 8.5% in winter wheat. The pathogenicity of *R. cerealis* isolates to wheat was confirmed in controlled environment experiments where significant damping-off and stem browning on 10 day old wheat seedlings occurred. In this study root rot was not correlated with any pathogen and was likely caused by a complex of species. Root rot incidence and severity in English wheat fields and in field experiments showed a decreasing trend in both seasons. BFR was the predominant SBD in English wheat crops occurring in 100% of crops by GS 37 in both seasons. Eyespot showed season to season variation and was favoured by warm, wet and humid conditions in the spring. The causal agents of eyespot the *Oculimacula* spp. were the most common occurring species in the soil and *in planta* of English wheat crops. DNA of *Oculimacula* spp. and *Microdochium* spp. was detected in ≥90% of stem bases in both seasons, showing these species are ubiquitous in English wheat crops. Results indicated a shift in the *Oculimacula* spp. population in English wheat crops with both species occurring together although *O. yallundae* was clearly the predominant species in terms of biomass.

Protecting plants in the establishment phase of development from *Rhizoctonia* spp. is critical as early infections can lead to seedling death. Seed treatments are considered the most effective way to target and deliver fungicides to control infections by *Rhizoctonia* spp. In field experiments sedaxane + fludioxonil provided more consistent control of damping-off and stem browning than fludioxonil alone. In addition, fungicide EC_{50} values to inhibit mycelial growth of *R. solani* and *R. cerealis* isolates were lower for sedaxane than fludioxonil or prothioconazole. Sedaxane + fludioxonil also significantly reduced BFR up to GS 39, but showed inconsistent control of sharp eyespot beyond GS 13.

To conclude, this study has found that AG 2-1 is widespread in English wheats crops and this may have implications for the growth and development of OSR.

Rhizoctonia cerealis was shown to significantly reduced emergence and grain yield of winter wheat. This suggests control of this pathogen should receive greater attention especially at the establishment phase of development and that seed treatments (i.e. sedaxane) are the most effective control option to protect seedlings.

Chapter 1

Literature Review

1.1 INTRODUCTION

Three crops wheat (*Triticum aestivum*, L.), rice (*Oryza sativa*) and maize (*Zea mays*, L.) dominate global cereal production, accounting for 90% of total cereal production and 60% of the world's food energy intake (FAOSTAT, http://faostat.fao.org/). In 2012 there was 671 million tonnes (Mt) of wheat produced compared to 739 Mt of rice and 872 Mt of maize (FAOSTAT, http://faostat.fao.org/). However, the ability of wheat to be cultivated in diverse environments is unmatched with crops grown from 67° N in Russia to 45° S in Argentina, in addition to areas of the tropics and sub-tropics (Feldman, 1995). Wheat was one of the first grains to be domesticated ~10,000 years ago (Shewry, 2009) and has been the staple food in the major civilizations of Europe, West Asia and North Africa for the last ~8000 years (Courtis, 2002). The most widely grown domesticated form accounting for 95% of wheat grown worldwide is the hexaploid bread wheat *Triticum aestivum* (Shewry, 2009). The hexaploid *T. aestivum* contains six sets of chromosomes (genome AABBDD) that has arisen from the hybridization of *T. dicoccoides* (genome AABB) (emmer) and the unrelated wild grass *Aegilops tauschii* Coss (genome DD) (goat grass) (Hanock, 2004; Dvorak *et al*., 2012). In the UK wheat is the most widely grown crop covering approximately 1.8 million ha with total production at ~18 Mt annually (DEFRA, 2015). Approximately 39% of the crop grown in the UK is for human consumption and industry usage (e.g. flour, starch or biofuels) and 42% destined for animal feed (AHDB, 2015). In 2015 the average UK wheat yield was 8.8 tonnes (t) ha-1 (DEFRA, 2015) which is relatively high compared to the global average of approximately three t ha^{-1} (Shewry, 2009). However, a combination of abiotic and biotic stresses continuously erodes the potential yield of wheat. In Northwest Europe biotic stresses including weeds, insects, animal pests and pathogens cause estimated yield losses of 14% in wheat with fungal diseases considered to have the greatest impact on yield due to the intensity of cropping (Oerke, 2006). Therefore, it is essential to understand which diseases and pathogens are occurring in wheat crops as this will inform management control strategies to reduce potential yield losses.

1.2 *Rhizoctonia*

Rhizoctonia species (phylum: *Basidiomycota*, class: *Basidiomycetes*, genera: *Thanatephorus, Waitea, Koleroga, Ceratobasidium, Uthatobasidium, Athelia* and *Botryobasidium*) are ubiquitous group of fungi causing a range of destructive diseases on many crop species (Ogoshi, 1996; Roberts, 1999). *Rhizoctonia* spp. are mainly associated with soil borne diseases affecting roots and stems of a vast range of crop species. However, saprophytic and symbiotic taxa also occur. *Rhizoctonia* spp. are distributed worldwide in agricultural and forest soils and are known to cause some of the most economically important diseases in agricultural crops (Agrios, 2005; Strange and Scott, 2005). The most widely studied groups of *Rhizoctonia* species are the multinucleate *Rhizoctonia solani* and the binucleate (BNR) *Rhizoctonia* species. *Rhizoctonia solani* Kühn [teleomorph synonym *Thanatephorus cucumeris* Donk] causes diseases in a wide range of economically important crops including rice (*Oryza sativa*), potatoes (*Solanum tuberosum*), maize (*Zea mays*) and wheat (*Triticum aestivum*) (Mazzola *et al.*, 1996; Ogoshi, 1996). The BNR species include many crop pathogens but perhaps *Rhizoctonia cerealis* Van der Hoeven [teleomorph synonym *Ceratobasidium cereale* (D Murray and LL Burpee)], the causal agent of sharp eyespot is the most well-known. *Rhizoctonia cerealis* preferentially infects wheat but also infects other cereals such as barley (*Hordeum vulgare*) and oats (*Avena sativa*) (Hamada *et al*., 2011a).

1.3 Biology of *Rhizoctonia* spp.

1.3.1 Classification

Based on vegetative characteristics *Rhizoctonia* spp. are grouped into the genus *Rhizoctonia.* Although dual nomenclature for pleomorphic fungi has been discontinued in 2013 (Hawksworth, 2011), the teleomorph and anamorph names and stages are briefly discussed below, with reference to the telemorph name where appropriate. The teleomorph (sexual perfect stage) is known for many anamorphs (asexual imperfect stage) of many *Rhizoctonia* spp. including *R. solani* and *R. cerealis.* The teleomorph of *R. solani* is *Thanatephorus cucumeris* (Frank) Donk and for *R. cerealis* is *Ceratobasidium cereale* (D Murray and LL Burpee). The teleomorphic stages of *Rhizoctonia* spp. have proven difficult to induce under laboratory conditions; therefore their characterisation has been mainly based on anamorphic features (García *et al.*, 2006). The main five attributes that are used to characterise *Rhizoctonia* spp. are –

1. Branching near the distal septum in young, vegetative hyphae
2. Hyphal constriction and septa formation located near the origin of branches
3. Dolipore septum
4. Lack of clamp connections, rhizomorphs or conidia
5. Undifferentiated sclerotia

(adapted from Ogoshi, 1987; Sneh *et al.*, 1991)

Thanatephorus and *Ceratobasidium* belong in the family *Ceratobasidiacea*, Order *Cantharellales*, Class *Basidiomycetes*, Phylum *Basidiomycota* (Roberts, 1999). *Thanatephorus* and *Ceratobasidium* are also morphologically closely related. However, *Thanatephorus* can be distinguished from *Ceratobasidium* by its vertically branching hymenium producing successive layers of basidia (Roberts, 1999). The basidia are also more elongated in *Thanatephorus* with a length to width ratio of 1.2 and 3.2, thus the average ratio is above 1.4 which is the maximum ration for *Ceratobasidium* (Roberts, 1999). *Thanatephorus cucumeris* and *Ceratobasidium cereale* are also distinguished by the number of nuclei present. All *Rhizoctonia* spp. hyphae are separated into sections by septa (Roberts, 1999). The number of nuclei present between the septa can be used as a simple method to identify between binucleate and multinucleate species

(Stalpers and Andersen, 1996). *Thanatephorus cucumeris* are multinucleate species, with each cell having at least three nuclei and *Ceratobasidium cereale* are binucleate species and have two nuclei per cell.

1.3.2 Lifecycle of *Rhizoctonia*

All *Rhizoctonia* spp. exist primarily as vegetative mycelium or as sclerotia. In their *anamorphic* stage *R. solani* and *R. cerealis* share some common features. The two main characteristics are the branching of growing hyphae at right angles and the lack of asexual spores (conidia). The asexual stage of the lifecycle is responsible for the production of vegetative mycelium or sclerotia that are considered the primary source of infection causing the majority of rhizoctonia diseases (Anderson, 1982; Cubeta and Vilgalys, 1997). Of these two sources of inoculum sclerotia are thought to incite the vast majority of plant diseases (Anderson, 1982). As previously mentioned in their *teleomorph* state the multinucleate *R. solani* is *Thanatephorus cucumeris.* However, some other multinucleate *Rhizoctonia* spp. that are plant pathogens (e.g. *R. zeae* and *R. oryzae*) have *Waitea circinata* as there *teleomorph* (Oniki *et al.,* 1985). The *teleomorph* of the binucleate *Rhizoctonia* spp. is known as *Ceratobasidium* with *R. cerealis* having the *teleomorph C. cereale.*

The sexual stage of *T. cucumeris* is not well understood and was previously thought to be a homothallic mating system (Kotila, 1929; Hawn and Vanterpool, 1953). However, studies in laboratory conditions where isolates have produced sexual spores have shown some isolates have heterothallic mating systems (Whitney and Parmeter, 1963; Adams and Butler, 1982). In addition, although only shown in a few AG's of *R. solani*, field isolates were found to be both homokaryotic and heterokaryotic. Isolates of AG 1-IC and AG 2 were homokaryotic and isolates of AG 1-IC, AG 4 and AG 8 were found to be heterokaryotic with a bipolar mating system (Adams and Butler, 1982; Anderson, 1982; Yang *et al*., 1992, Julian *et al*., 1996 and Julian *et al*., 1997). More recently Toda and Hyakumachi (2006) found homothallic and heterothallic field isolates of *T. cucumeris* AG 2-2-IV were present in the population and that genetic exchange can occur between homothallic and heterothallic isolates. Mating

between a homokaryotic and heterokaryotic parent is likely to increase the genetic diversity within field isolates of *R. solani* offering a fitness advantage (Qu *et al.* 2013). The mating system of the binucleate *C. cereale* is not known but could be hypothesised to be the similar to that of *T. cucumeris.* The name *Rhizoctonia solani* and *Rhizoctonia cerealis* will now be used throughout this study.

1.3.2 Hyphal interactions in *Rhizoctonia*

Classification of *Rhizoctonia* spp. has traditionally been based on the ability of hyphae to anastomose. Anastomosis can simply be defined as the fusion of hyphal pairings between two separate *Rhizoctonia* isolates. Hyphal paring is generally performed with a tester isolate of a known AG's and possibly subgroups (Naito, 2006). Four possible anastomosis reactions are known to occur between the paired hyphae. Previous authors have established a set of terminology to describe the four reactions (Table 1.1) which are observable at the light microscope level. The most widely adopted terminology is that of Carling *et al*. (1988). Carling *et al*. (1988) applied categories (C) to the four anastomosis reactions. Within this system C0 indicates no fusion (contact) therefore the hyphae are from different AG groups and not genetically related. The C1 reaction is when hyphae fuse but show no obvious wall penetration or membrane contact, implying the AGs are from different groups (Carling, 1996) (Figure 1). The remaining C2 and C3 reactions are most relevant regarding the classification of isolates into AGs. Category 2 reaction demonstrates obvious wall connection but uncertain membrane fusion, in time the anastomosing and adjacent cells die (Carling *et al*., 1988; Carling, 1996). This is also commonly referred to as the 'killing reaction' and refers to genetically distinct isolates belonging to the same AG (Cubeta and Vilgalys, 1997). The C3 reaction, also referred to as 'perfect fusion', shows clear wall and membrane fusion with limited if any death of anastomosing cells, point of fusion width is generally equal to hyphal width (Carling *et al*., 1988; Carling, 1996). Therefore, C3 reaction indicates genetic or near genetic relatedness between isolates. Isolates which undergo perfect fusion with each other belong to the same vegetative compatibility group (VCG).

Table 1.1 Previous categorisation systems for anastomosis reactions between *Rhizoctonia* isolates (adapted from Carling, 1996)

Present Class (Carling *et al.*, 1988)	**Matsumoto *et al.* (1932)**	**Flentje *et al* (1964)**	**Parmeter *et al.* (1969)**
C0	No reaction	NR (No reaction)	0
C1	Contact	WF (Wall fusion)	1
C2	Imperfect	K (Killing)	2-Imperfect
C3	Perfect	S (Self)	2-Perfect

1.3.3 Anastomosis groups of binucleate and multinucleate *Rhizoctonia* spp.

To date the classification of multinucleate and binucleate species has been based on the grouping of isolates into the aforementioned anastomosis groups (AGs) (Table 1.1) (Carling, 1996). Cubeta and Vilgalys (1997) stated that "Anastomosis grouping still represents the single most important advance in our understanding of the genetic diversity within *Rhizoctonia*." Within the multinucleate *R. solani* species complex 13 AGs have been identified (Carling *et al.*, 2002a). In addition, subgroups within the *R. solani* complex have been identified for AG-1, -2, -3, -4, -6, -7, -8 and -9 (Ogoshi, 1987; Carling *et al.*, 2002b). The AG showing the most diversity is AG 2 with seven subgroups (2-1, 2-2 IIIB, 2-2 IV, 2-2 LP, 2-3, 2-4 and 2-BI) (Carling *et al*. 2002b). Each *R. solani* AG and subgroup are able to infect a diverse range of crops (Table 1.2). Isolates of the binucleate *Rhizoctonia* species are also assembled in anastomosis groups (AGs) but instead of numbers to distinguish between groups letters are used. To date there are 21 binucleate AG's of the genus *Ceratobasidium* that have been named AG-A to AG-U (Ogoshi *et al.*, 1983; Sneh *et al.*, 1991; Hyakumachi *et al.* 2005). Although some groups have been excluded such as AG-T, that actually belonged to AG-A, by reinvestigations using molecular phylogeny (Sharon *et al.*, 2008). *Rhizoctonia cerealis* is assigned to AG-D and within this species three subgroups (I, II, III) have been identified (Toda *et al.* 1999; Hayakawa *et al.*, 2006). Research suggests AG D-I is the main sharp eyespot causing species (Li *et al.* 2014). Binucleate *Rhizoctonia* spp. and subgroups are also able to infect a diverse range of crops (Table 1.3).

Table 1.2 Summary of the different groups and subgroups recognized for multinucleate *Rhizoctonia solani* and their hosts

AG	Subgroup	Host range	Reference
AG 1	IA	Rice, corn, sorghum, bean, soybean, crimson, clover, camphor seedlings, turfgrass	Ciampi *et al*. (2008); Naito (2004); Sneh *et al*. (1998)
	IB	Sugarbeet, cabbage, rice, bean, soybean, snap bean, corn, cabbage, lettuce, turfgrass	Naito (2004); Sneh *et al*. (1998)
	IC	Sugarbeet, buckwheat, carrot, flax, soybean, pine; cabbage, radish, spinach	Naito (2004); Ogoshi (1987); Sneh *et al*. (1998)
	ID	Coffee	Priyatmojo *et al*. (2001)
AG 2	1	Pea, cabbage, radish, broccoli, tulip, lettuce, canola, lupin, medic, clover, wheat, potato, soybean, strawberry	Campion *et al.* (2003); Tewoldemedhin *et al*. (2006); Truter and Wehner (2004); Tewoldemedhin and Lamprecht (2013)
	2-IIIB	Soybean, potato, maize, St. Augustine grass and *Zoysia* grass	Muzhinji *et al*. (2015); Priyatmojo *et al*. (2001); Strausbaugh *et al*. (2011);
	2-IV	Sugerbeet, turf, carrot, eggplant, pepper, spinach, stevenia	Salazar *et al*. (2000); *Sneh et al*. (1998); Naito (2004)
	2-LP	Zoysia grass	Aoyagi *et al*. (1998)
	3	Soybean	Salazar *et al*. (2000)
	4	Corn and carrot.	Sumner (1985)
AG 3	PT, TB, TM	Solanaceae (tobacco, tomato, potato), pepper, lupin, canola, wheat, sugarbeet	Truter and Wehner (2004); Tewoldemedhin *et al.* (2006); Sneh *et al*. (1998)
AG 4	HG-I, HG-II, HG-III	Peanut, sugarbeet, pea, spinach, potato, onion, snap bean, tomato, melon, turfgrass, zinnia, poinsettia, carnation, hibiscus, soybean, barley, canola, medic, clover, wheat	Tewoldemedhin and Lamprecht (2013); Muzhinji et al. (2014); Sneh et al. (1998); Kuramae et al. (2003), Naito (2004)
AG 5		Soybean, potato, bean, turfgrass, mycorrhizal association, couch grass, wheat	Naito (2004); Sneh *et al*. (1998); Truter and Wehner (2004), Woodhall and Lees (2004). Woodhall *et al.*, (2012b)
AG 6	I, V	Turfgrass, mycorrhizal association, lucerne, apple, wheat, strawberry	Mazzola (1997); Botha *et al*. (2003); Meyer *et al*. (1998)
AG 7		Potato, cotton, soybean, radish, and rice	Truter and Wehner (2004); Rothrock *et al*. (1993)
AG 8		Barley, yellow mustard, safflower, canola, wheat, oats, triticale, clover, lucerne.	MacNish and Neate (1996); Truter and Wehner (2004)
AG 9	TP, TX	Crucifers and potato, carrot, lettuce (mildly parasitic),	Naito (2004); Sneh *et al*. (1998);Yang *et al*. (1996)
AG 10		Barley, lettuce, tomato (weak pathogen), canola, wheat	Khangura *et al.*(1999); Sneh *et al*. (1998)
AG 11		Rice, Lupin, barley, lupine, soybean, and wheat	Kumar *et al*. (2002); Tewoldemedhin *et al*. (2006)
AG 12		Lettuce, cauliflower, radish seedlings.	Carling *et al.* (1999)
AG 13		Cotton, radish, cauliflower (weak pathogen)	Carling *et al*. (2002a)

Adapted from: Muzhiji (2016) PhD thesis Pretoria University.

Table 1.3 Summary of the different groups and subgroups recognized for binucleate *Rhizoctonia* spp. and their hosts

AG	Subgroup	Host range	References
AG A		Potato, canola, lucerne, barley, medic, apples, soybean, potato, strawberry	Muzhinji *et al.* (2015); Tewoldemedhin *et al.* (2006); Tewoldemedhin and Lamprecht (2013)
AG B	$B_{0,}$ Ba, Bb	Canola, lupin, rice	Ogoshi *et al*. (1996)
AG C		Strawberry	Fang *et al.* (2013)
AG D	I, II, III	*Poaceae*	Toda *et al.* (1999); Hayakawa *et al.* (2006)
AG E		*Erigeron*	Burpee *et al.* (1980a)
AG F	Fa, Fb	Soybean	Tewoldemedhin and Lamprecht (2013)
AG G		Apples, strawberry, peanut	Tewoldemedhin *et al.* 2011; Fang *et al.* (2013)
AG H		Soil	Ogoshi *et al*. (1979)
AG I		*Artemisia*	Ogoshi *et al*. (1979)
AG K		Canola, lucerne, lupin, canola, strawberry, Sugar beet	Tewoldemedhin *et al.* (2006); Fang *et al.* (2013)
AG L		Soil	Ogoshi *et al*. (1983)
AG O		Soil	Ogoshi *et al*. (1983)
AG P		Tea	Ogoshi *et al*. (1983)
AG R		Potato	Muzhinji *et al.* (2015)
AG S		*Pittosporum*	Burpee *et al.* (1980a)
AG U		Cut-flower roses	Hyakumachi *et al.* (2005)
AG V		Ginger, taro	Yang *et al.* (2013)
AG W		Potato	Yang *et al*. (2015)
Unidentified BNR		Potato, wheat, rooibos	Tewoldemedhin *et al.* (2006, 2015); Woodhall *et al*. (2011)

Adapted from: Muzhiji (2016) PhD thesis Pretoria University.

1.4 Detection and identification of *Rhizoctonia* spp. and anastomosis groups

Reliable diagnostic techniques are vital for the correct identification and classification of *Rhizoctonia* spp.

1.4.1 DNA sequencing

Sequencing of ribosomal DNA (rDNA) is a powerful method for identifying *Rhizoctonia* spp. (Kuninaga *et al.,* 1997; Toda *et al.,* 2004; Sharon *et al.,* 2006 and 2008). rDNA consists of gene segments of which the internal transcribed spacer region has shown a wide degree of variation in fungi (Schoch *et al.,* 2012). The heterogeneity of the rDNA-ITS in *Rhizoctonia* spp. has enabled the accurate identity of species and subgroups (Kuninaga *et al.,* 1997; Hayakawa *et al.,* 2006; Sharon *et al.,* 2006; Sharon *et al.,* 2008). Sharon *et al.,* (2006) stated that sequencing of rDNA-ITS is the most appropriate method for the classification of *Rhizoctonia* spp. and that it generally supports previous AG's and subgroups based on 'classical' hyphal fusion. Although sequencing of rDNA-ITS may not be appropriate for all AGs. For example, isolates of AG-K and AG-A do not anastomose which may indicate they are distinct genetic groups but there is some overlap in percent sequence similarity range (Sharon *et al.,* 2008).

1.4.2 Nucleic acid amplification

1.4.2.1 Conventional PCR

Conventional PCR assays can be used to identify *Rhizoctonia* spp. but are a poor method to quantify pathogen DNA and also require post amplification steps such as gel electrophoresis and ethidium bromide staining. Additionally, the use of conventional PCR may not be a suitable method to amplify pathogen DNA extracted from soil (Lees *et al.,* 2002).

1.4.2.2 Real-time PCR

The development of real-time PCR assays based on the sequence of the rDNA-ITS region are species specific and have allowed the unambiguous identification of the target pathogen but also accurate quantification in soil and plant material (Budge *et al.,* 2009; Okubara *et al.,* 2008; Hamada *et al.,* 2012; Woodhall *et al.,*

2013). Real-time PCR assays are quicker and less time consuming than conventional PCRs. In addition the sensitivity of real-time PCR is known to exceed that of conventional PCR and ELISA by 100 and 1000 times respectively (Ratti *et al.,* 2004). Increased sensitivity of assays has allowed specific sub-groups to be targeted. This is advantageous when identifying subgroups of the same AG that have different host ranges, for example AG 3-PT infects potatoes but AG 3-TB is a pathogen of tobacco (Woodhall *et al.,* 2013). Real-time PCR assays generally utilise SYBR green and TaqMan chemistries. SYBR green assays are advantageous due to their simple design, easy set up and lower cost. TaqMan assays, with an additional labelled probe, have the advantage of high specificity and reproducibility but are more expensive. There are several reports of SYBR green assays (Sayler and Yang, 2007; Okubara *et al.,* 2008; Guo *et al.,* 2012) and TaqMan assays (Lees *et al.,* 2002; Budge *et al.,* 2009; Woodhall *et al.,* 2013). However, one of the main advantages of Real-time PCR is the accurate quantification of species. This has also led to a greater understanding of the predominant *Rhizoctonia* spp. and how environmental and agronomic factors influence these species (Okubara *et al.,* 2014; Sparrow *et al.* 2015). Furthermore loop-mediated isothermal amplification assays (LAMP) could provide a simple and cheap method for identifying pathogens in the field (Tomlinson *et al.,* 2010). This would allow growers to identify individual AG's and therefore establish the risk to crops and tailor specific control management strategies. To date there has only been a limited number of LAMP assays developed to detected *Rhizoctonia* spp. and none so far are specific to individual AGs (Lu *et al.,* 2015; Patel *et al.,* 2015).

1.4.3 Detection in soil

1.4.3.1 Plating onto selective media

Traditionally culturing isolates onto selective media was the main method to estimate soil populations (Ko and Hora, 1971; Doornik, 1981; Vincelli and Beaupré, 1989). The most commonly used media is that developed by Ko and Hora, (1971). This method has been adapted by various researchers for example, Castro *et al.* (1988) suggest the addition of prochloraz to improve the recovery of

Rhizoctonia spp. However, plating is a laborious process and has now largely been superseded by biochemical and molecular methods.

1.4.3.2 Baiting

Baiting methods can be used to isolate viable *Rhizoctonia* spp. from soil. The two most successful methods are the wooden toothpick and seed baiting methods (Paulitz and Schroeder, 2005; Boine *et al.*, 2014; Spurlock *et al.*, 2015). The toothpick method enables not just the isolation of viable isolates but also quantification of species in soil (Paulitz and Schroeder, 2005). Boine *et al.* (2014) successfully used seed (quinoa) baiting with real-time PCR assays to investigate the impact of host and non-host plant residues on AG 2-2IIIB levels in the soil. However, seed baiting is likely to lead to the amplification of *Rhizoctonia* spp. as a food source is provided and therefore the amount detected can be higher than actual soil levels (Lees *et al.*, 2002). Also baiting can be selective favouring the most competitive saprophytic species. For example, a range of multinucleate and binucleate species were isolated but no *R. cerealis* from soil samples collected across Europe using seed and toothpick baiting methods (Goll *et al.*, 2014). However, this method is still important to collect viable isolates from soil samples of known and unknown *Rhizoctonia* spp. for further characterisation work.

1.4.3.3 Enzyme-linked immunosorbent assay (ELISA)

Enzyme-linked immunosorbent assay (ELISA) that utilises the antigen-antibody binding interaction has been used to detect *Rhizoctonia* spp. Thornton *et al.* (1999) combined soil baiting using quinoa seed with a double monoclonal antibody ELISA assay. This method had the advantage of allowing the recovery of live *R. solani* isolates from the seed after the ELISA assays, therefore allowing further characterisation studies (e.g. pathogenicity trials). However, the method is limited as it cannot reliably distinguish between isolates of different AGs and cross reactivity between fungal species can occur. ELISA is far less sensitive than nucleic acid-based techniques (Ward *et al.*, 2004).

1.4.3.4 Soil DNA extractions and PCR

Rhizoctonia spp. are soil borne fungi therefore accurate identification and quantification in soil is important to understand the populations dynamics in soil. DNA extracted from soil has generally been obtained from relatively small soil samples (0.5 g to 60 g) (Cullen *et al.,* 2001; Lees *et al.,* 2002; Brieley *et al.,* 2009; Okubara *et al.,* 2014) which are not representative of the population due to the random distribution of *Rhizoctonia* spp. in fields. The development of extraction methods from large volumes of soil of up to1 kg has enabled quantification from more representative sample (Woodhall *et al*., 2012a; Sparrow *et al.,* 2015). DNA extractions from large volumes of soil can detect one 200 µg sclerotia in a 250 g sample which is the equivalent to 8×10^{-7} g sclerotia g^{-1} of soil (Woodhall *et al.,* 2013). This shows the sensitivity of large soil extractions. However, one limitation of soil DNA extraction is that non-viable DNA can be extracted. This can be overcome to some extent by combining seed baiting and PCR (Lees *et al.,* 2002; Boine *et al.,* 2014).

1.5 Rhizoctonia diseases in wheat

1.5.1 Damping-off and root rot caused by *R. solani*

Various AG's of *R. solani* can infect the roots and/or stems of wheat plants (see section 1.7). In the establishment phase of development the pathogen can cause pre- and post-emergence damping-off (Tewoldemedhin *et al*. 2006) leading to a reduction in plants numbers. However, rhizoctonia root rot is the classic symptom associated with *R. solani* in wheat production. Symptoms observed on roots are brown sunken or necrotic lesions, which may girdle the root circumference severing the root. This is referred to as 'spear-tipping' which leaves a severely pruned appearance (Figure 1.1) (Weller *et al.,* 1986; Mazzola *et al.,* 1996). In addition, primary infections can lead to roots been predisposed to secondary infections caused by a range of soil-borne fungi (Harris and Moen, 1985; Anees *et al.,* 2010). However, identifying infected roots in the field can be problematic due to the destructive nature of removing plants from soil for visual assessment. Cook *et al.* (2002) also stated that lesions on roots caused by other diseases such as take-all (*Gaeumannomyces graminis* var. *tritici*) can lead to an underestimation of the severity of rhizoctonia root rot present. Above ground symptoms generally manifest in severely stunted plants that occur in distinct patches, the size of patches are probably related to the growth and distribution of *R. solani* within soil (Weller *et al.,* 1986). The stunted plants resemble those under stressed conditions such as drought or poor nutrition (MacNish and Neate, 1996). This can lead to the misdiagnosis of the cause of stunted growth.

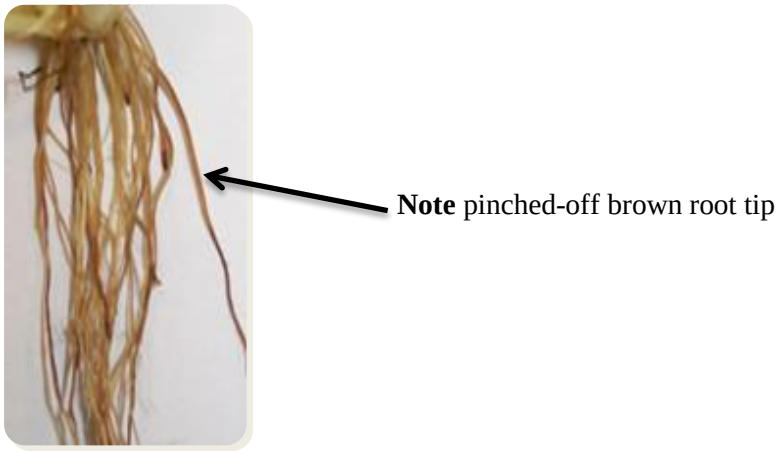


Figure 1.1 Rhizoctonia root rot on wheat roots

1.5.2 Sharp eyespot caused by *R. cerealis*

Rhizoctonia cerealis is the causal agent of sharp eyespot that occurs on the stems of wheat plants. The classic sharp eyespot symptoms are lesions on the stem that have a pale cream centre with a dark brown, sharply defined edge (Figure 1.2). The lesions act by destroying the phloem tissues in the leaf sheath and stems blocking the nutrient transport system that can lead to whiteheads, lodging and ultimately loss of yield. However, in the early stages of crop development sharp eyespot can be difficult to identify due to mixed symptoms caused by a complex of stem base pathogens. Other common stem base diseases include eyespot caused by *Oculimacula acuformis* and *O. yallundae* and brown foot root caused principally caused by *Microdochium* spp. but also *Fusarium* spp. (Polley and Turner, 1995; Bateman *et al.,* 2000). The occurrence of these three diseases together is known as the stem bases disease (SBD) complex. Correct identification of these individual diseases in the early growth stages of cereals can be confounded, especially when differentiating sharp eyespot from eyespot (Daamen and Stol, 1990). However, correct identification of sharp eyespot is more successful at later growth stages (GS 39>) when symptoms are clearly visible (Turner *et al.,* 2001).

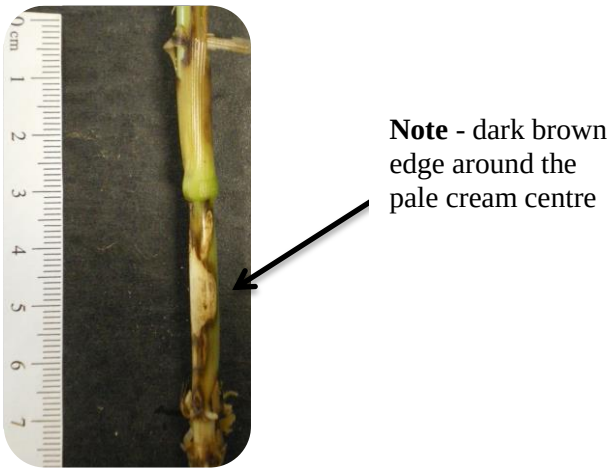


Figure 1.2 Sharp eyespot symptoms on wheat stems

1.5.3 Yield losses due to rhizoctonia diseases in wheat

Rhizoctonia solani can cause yield losses in wheat production. For example, rhizoctonia root rot has been highlighted as a disease of major concern in Australia (MacNish and Neate, 1996). However, published literature that has quantified actual within field yield losses in wheat caused by *R. solani* are limited and generally focused on AG 8. MacNish, (1985) reported that yield within bare patches caused by rhizoctonia root rot were 8% lower than those outside of bare patches. Nonetheless overall yield loss in Australia due *R. solani* in wheat has been estimated at only 2% in Southern Australia, 1.1% in Western Australia and 0% in Northern Australia (Murray and Brennan, 2009). However, losses of 25% or more have been cited in the literature (MacNish and Neate, 1996). Yield losses in Australia to a range of soil borne pathogens, including *R. solani* AG 8, have reached such an extent that since 1997 a routine soil DNA-based testing service has been available (Ophel-Keller *et al.*, 2008). This allows growers/agronomists to predict losses from various soil borne pathogens (Ophel-Keller *et al.*, 2008). In the UK, such a service is not available and except for *R. cerealis* yield losses from other *Rhizoctonia* spp. pathogenic to wheat are not known.

Yield losses in wheat due to sharp eyespot are mainly caused by severe infections, although moderate infections can also cause yield losses (Hamada *et al.*, 2011a). Yield losses in the UK over five seasons observed by Clarkson and Cook (1983) were 5% for moderate infection and 26% for severe infections. Lemańczyk and Kwaśna, (2013) in Poland also showed that with increasing severity of sharp eyespot a decrease in a number of grain yield (e.g. grain dry weight/ear) and grain quality (e.g. thousand-grain weight) components was recorded. Further afield mean losses in 43 fields in New Zealand over a four year period were estimated at 6, 1, 8, and 14% for each year (1996-2000) of the survey (Cromey *et al.*, 2002). The authors also found yield losses were related to disease incidence with no notable losses when incidence was <10%. In China sharp eyespot is regarded as serious threat to wheat production with losses estimated at over one billion Yuan from 2005 to 2008 (Chen *et al.*, 2008). These reports demonstrate the significance of sharp eyespot, on wheat production.

1.6 Disease cycle of *R. solani* and *R. cerealis*

The lifecycle of *R. solani* AG's and *R. cerealis* are considered to be similar enough to allow generalised description to cover the disease cycle of both species (García *et al.* 2006). The main difference in the disease cycle is that *R. solani* is generally considered a root pathogen and *R. cerealis* a stem pathogen. Figure 1.3 shows the basic disease cycle of *R. solani* on wheat. The disease cycle of *R. cerealis* on wheat is shown in Figure 1.4. In between hosts both species survive as sclerotia (dense asexual fungal resting structures) or as mycelium that exists saprophytically on dead and decaying organic matter in the soil. Root exudates released by actively growing plant cells of a host species activate the pathogen to send out runner hyphae (Dodman and Flentje, 1970). The ability of the hyphae to spread and infect a host depends on the distance between nutrient sources and to some extent soil structure (Bailey *et al.,* 2000; Schroeder and Paulitz, 2008). Once contact is made hyphae spread along roots or stems forming dome shaped infection cushions (García *et al.,* 2006). Beneath the infection cushion an infection peg directly penetrates through the cuticle and epidermal cell walls releasing hydrolytic enzymes that degrade the plants cell walls (e.g. cellulose, cutin and pectin). This releases nutrients to sustain the pathogens growth and development. Damaged or dead plant cells manifest as the visual symptoms that develop on the roots or stems. As the plant senesces, nutrient sources become exhausted or environmental conditions become unconducive to the survival of the pathogen the hyphae mass together to form sclerotia (see section 1.6.3). Sclerotia can survive in the soil for a number of years until appropriate conditions or a host is present for disease cycle to start again. However, both species can also survive as saprophytes in the soil on crop debris, autumn sown hosts and volunteers and on weeds.

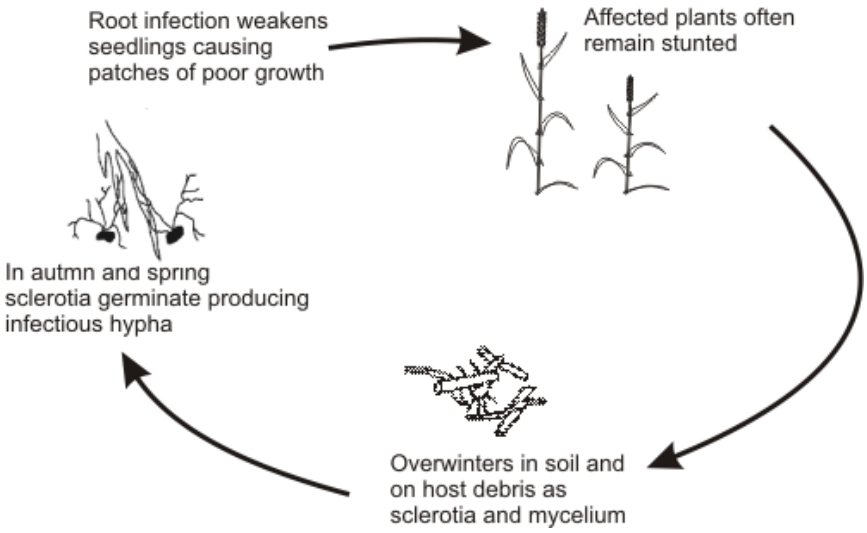


Figure 1.3 Disease cycle of rhizoctonia root rot on wheat

(Source AHDB, not dated(a))

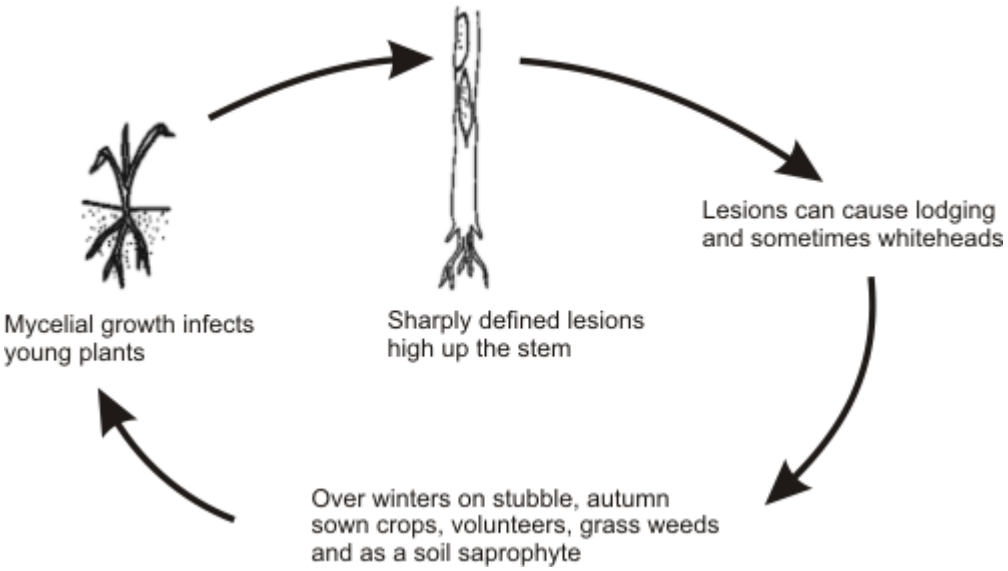


Figure 1.4 Disease cycle of sharp eyespot caused by *R. cerealis*

(Source adapted from AHDB, not dated(b))

1.6.2 The role of basidiospores

Basidiospores (reproductive spores) are produced by some *Rhizoctonia* spp. There are no reports of basidiospores causing disease in wheat. However, basidiospores do cause foliar diseases in some host crops, for example foliar blight of sugar beet is attributed to basidiospores of the teleomorph stage of *R. solani* AG-2-2 IV (Naito, 1996). Similarly, target spot disease of tobacco and tomato foliar blight is caused by basidiospores of *R. solani* AG 3 (Jonk *et al.* 1993; Bartz *et al.* 2010). Production of basidiospores seems to be initiated under warm (≥20°C) and humid (≥90% relative humidity) conditions with dispersal occurring in periods of darkness (Naito, 1996, Naito, 2006). However, the role of basidiospores in disease epidemiology is yet to be clearly established (Naito, 2006). It is generally considered that basidiospores are not the primary source of inoculum in most disease systems (Cubeta and Vilgalys, 1997). Although the growing number of reports of basidiospores causing disease in a number of plants suggests the role of these reproductive spores is underestimated in the disease cycle of this pathogen.

1.6.3 Survival of inoculum

The main survival mechanism for both *R. solani* and *R. cerealis* is the formation of sclerotia allowing the pathogen to survive in the absence of a host within organic matter or debris in the soil (Papavizas *et al*., 1975; Lipps and Herr, 1982). Sclerotia also allow the pathogens to survive periods of adverse environment condition, a lack of nutrients or competition from microbial populations. Sclerotia are formed primarily by compacted masses of monilioid cells which are small (1-3 mm in diameter) in size and of irregular shape and generally brown/black in colour. This tightly compacted structure and the presence of melanin in sclerotia cells walls protects the pathogen against environmental stresses (e.g. microbial attack) (Sneh, 1996; Sumner, 1996). Furthermore, the sclerotia of *R. solani* commonly exude a brown liquid that contains phenolics, carboxylic acids, carbohydrates, fatty acids and amino acids that deter predators and express anti-fungal properties (Aliferis and Jabaji, 2010). It is hypothesised that sclerotia of *R. cerealis* also produce similar substances (Coley-Smith and Cooke, 1972). The movement of sclerotia on plant material (e.g. straw) or in soil (e.g. on farm machinery) will be two of the main mechanisms by which the pathogen is spread.

1.6.4 Effect of temperature and moisture on disease development

The occurrence of severe disease in wheat caused by *R. solani* and *R. cerealis* is dependent on the presence of favourable environmental conditions, especially moisture and temperature.

There are limited studies on the effect of temperature and moisture on rhizoctonia root rot of wheat apart from *R. solani* AG 8. Root damage caused by AG 8 was greater at cooler soil temperatures (19°C day 6°C night) than at higher soil temperatures (27°C day 16°C night) (Smiley and Uddin, 1993). This was supported by Gill *et al.* (2001b) who found that disease caused by AG 8 was most severe at 10°C, explaining that disease suppression probably occurred in soil at higher temperatures due to greater antagonistic microbial activity. Smiley *et al.* (1996) observed in field studies that Rhizoctonia root rot was favoured in dry years. This was supported by Gill *et al.* (2001a) in a controlled environment study investigating root rot caused by AG 8 with soil moisture levels between 15 to 75% WHC. These workers reported that disease was higher in dryer soils compared to wet soils. They suggested that at low moisture levels roots exudates attracted the pathogen while at high moisture levels increased antagonistic microbial activity suppressing the pathogen. The effect of temperature and moisture of other AG's that have shown some pathogenicity to wheat has only been established on other crops. For example, Schneider *et al.* (1997) demonstrated that isolates belonging to AG 4 and AG 5 caused disease symptoms on bulbous crops at 18°C but none at 9°C. In an irrigated field study increasing soil moisture content resulted in significantly more disease in canola caused by AG 2-1 than AG 4 (Teo *et al.*, 1988). This shows the contrasting effects of temperature and moisture on diseases caused different AGs of *R. solani*.

Sharp eyespot infections can occur throughout the growing season but it is suggested the optimal temperatures for infection is around 9-10°C (Wiese, 1987; Parry, 1990). Hermen *et al.* (1992a) in a growth chamber study found sharp eyespot symptoms appeared earlier at 10°C and 15°C than 5°C, 18°C or 20°C. In controlled environment conditions Burpee *et al.* (1980b) observed the severity of sharp eyespot on wheat increased as soil temperatures decreased from 28°C to 16°C. Herman *et al.* (1992b) showed that soil moisture influenced sharp eyespot with disease incidence decreasing with increasing soil moisture content on 34 day

old wheat seedlings. While Kalberer and Gisi, (1997) concluded that the *R. cerealis* did not withstand very wet or very dry soil conditions. This would suggest the temperate conditions that occur in the UK are conducive to this disease.

1.7 Pathogenicity of *Rhizoctonia* spp. to wheat

The two most commonly associated pathogenic *Rhizoctonia* spp. to wheat are *R. solani* AG 8 and *R. cerealis*. However, there are reports of a number of other *Rhizoctonia* spp. causing disease in wheat.

1.7.1 *R. solani* AG 2-1

There has been contrasting reports of *R. solani* AG 2-1 as a pathogen of wheat. Roberts and Sivasithamparam (1986) isolated AG 2-1 from symptomatic wheat roots from within bare patches in Australia. In a controlled environment study these AG 2-1 isolates were able to cause significant root disease. In Turkey, AG 2-1 was isolated from the crown and subcrown internode of wheat plants and found to be moderately pathogenic on 30 day old seedlings (Demirci 1998). Sweetingham *et al.* (1986) isolated AG 2-1 from wheat and lupin plants and was shown to cause minimal disease on 20 day old wheat seedlings. Similarly Tewoldmedhin *et al.* (2006) in South Africa isolated AG 2-1 from a range of crops and found isolates to be non-pathogenic on wheat. Khangura *et al.* (1999) also showed that isolates of AG 2-1 isolated from OSR roots were non-pathogenic to wheat. However, a recent study utilising computed tomography (CT) to visualise the effect of AG 2-1 on the root architecture of wheat and OSR over time. Results of visual observations showed wheat roots were asymptomatic. However, CT scans revealed root architecture of wheat showed significant changes in the number of primary roots, root volume and root surface area (Sturrock *et al.*, 2015).

1.7.2 *R. solani* AG 2-2

R. solani AG 2-2 (of unknown subgroup) was shown to be highly virulent on 14 day old wheat seedlings causing significant damping-off in one study (Tewoldmedhin *et al.*, 2006). However, another study showed AG 2-2 (of unknown subgroup) had no effect on emergence and was only weakly pathogenic towards roots of seven day old wheat seedlings (Rush *et al.*, 1994).

1.7.3 *R. solani* AG 3

R. solani AG 3 (of unknown subgroup) has been shown to cause discolouration on the roots of wheat 60 days after inoculation (Carling *et al.*, 1986). Demirci (1998) showed that AG 3 was moderately virulent on the crown and subcrown

internode of 30 day old wheat seedlings. However, AG 3 is generally not considered a wheat pathogen but it may form an epiphytic interaction which could make crops such as wheat a poor rotational crop with potatoes (Carling *et al.,* 1986).

1.7.4 *R. solani* AG 4

Ogoshi *et al.* (1990) observed AG 4 not to cause root rot symptoms on wheat. In contrast Tewoldmedhin *et al*. (2006) found AG 4 (HG-II) to cause significant damping off and coleoptile rot on 14 day old wheat seedlings. The disparity in these two studies could be due to the difference in sub-groups. Ogoshi *et al.* (1990) did not distinguish which subgroup of AG 4 was used. There are three AG 4 sub-groups identified to date, HG I, II and III (Johnk and Jones, 2001) and it is highly likely each sub group differs in it pathogenicity to wheat.

1.7.5 *R. solani* AG 5

R. solani AG 5 was isolated from brown lesions on wheat stems taken from a field in Shropshire, England. Isolates were then used to inoculate wheat in a controlled environment study, after three weeks growth brown lesions were observed on seedlings. AG 5 was re-isolated from the brown lesions and identified as AG 5, there by confirming Koch's postulates (Woodhall *et al.,* 2012). Demirci *et al.* (1998) also showed that AG 5 caused moderate symptoms on the crown and subcrown internode of 30 day old wheat seedlings. On seven day old wheat seedlings Rush *et al.* (1994) observed significant root rot caused by AG 5. However, soil inoculated with AG 5 and incubated for two weeks prior to the sowing of wheat caused no root rot symptoms or any reductions in plant growth seven days after sowing (Ogoshi *et al.,* 1990).

1.7.6 *R. solani* AG 8

R. solani AG 8 is considered to be the main anastomosis group affecting wheat. It is a major pathogen of wheat in the Pacific Northwest of America and in Australia (Ogoshi *et al.,* 1990; MacNish and Neate, 1996). Infection results in the characteristic 'spear-tipping' or 'pinched-off' symptoms on roots leaving a severely pruned appearance (Weller *et al.,* 1986; Mazzola *et al.,* 1996). This manifests in the classic above ground bare patch symptoms (Weller *et al.,* 1986). However, the transitory nature and non-uniform distribution of bare patches

within fields (Cook *et al.*, 2002) makes yield losses due to AG 8 difficult to predict. Gill *et al.* (2002) showed AG 8 reduced dry weight of roots and shoots of 24 day old wheat seedling by 85% and 75% respectively in a controlled environment study. However, these results were only found in pots where the pathogen was allowed to colonise the soil for 10 days prior to sowing. When *R. solani* AG 8 was introduced at the time of sowing or 10 days post-sowing it failed to cause significant reductions in root and shoot weight. Gill *et al.* (2002) stated that disease at the time of sowing is pre-determined by the extent to which hyphal network is developed within the soil. This is likely to also apply to other AGs also pathogenic to wheat.

1.7.7 *R. solani* AG 11

The only other AG of *R. solani* shown to be pathogenic on wheat is AG 11. Demirci (1998) observed AG 11 to be highly virulent on wheat seedlings. However, other studies have shown the pathogen to cause only slight damage to roots or to be non-pathogenic (Carling *et al.*, 1994; Tewoldmedhin *et al*., 2006).

The aforementioned studies show there is much variation in the pathogenicity of isolates from the same AG of *R. solani* towards wheat. Differences in the pathogenicity of isolates of the same AG have been shown in other crops. For example, Woodhall *et al.* (2007) showed large variability in the ability of seven AG 2-1 isolates to cause disease on roots and stems of potatoes. The variation seen could be due to genetic isolation where isolates have evolved in separate geographical locations leading to the emergence of new strains with differing aggressiveness and host specificity. It has also been proposed that there is a complex of soil borne pathogens infecting the roots of wheat including one or more anastomosis groups of *Rhizoctonia* spp. along with other associated fungi (Harris and Moen, 1985; Sweetingham *et al*., 1986; Roberts and Sivasithamparam, 1986). Further research is required to investigate the interaction between species, particularly the effect of multiple soil borne pathogens on the yield of field grown wheat.

1.7.8 Binucleate *Rhizoctonia* spp.

Apart from *R. cerealis* there is very little information of other binucleate *Rhizoctonia* spp. as pathogens of wheat. The few studies that have been conducted have found binucleate *Rhizoctonia* spp. AG C, AG E, AG I and AG K to be non-pathogenic towards wheat (Roberts and Sivasithamparam, 1986; Windels and Nabben, 1989; Yang *et al.*, 1994; Demirci, 1998).

1.7.9 *Rhizoctonia* spp. in UK wheat fields

There is surprisingly little information regarding the population dynamics of *Rhizoctonia* spp. in UK wheat fields or arable fields in general. A survey of British potato crops between 2001 to 2003 showed that of the 135 *R. solani* isolates collected from infected plant material 92% were AG 3-PT; also found were AG 2-1 (6.7%) and AG 5 (0.7%) (Woodhall *et al*., 2007). However, the aim of the survey was to target *Rhizoctonia* spp. pathogenic to potatoes and therefore unlikely to identify other non-host *Rhizoctonia* spp. A less specific survey by Goll *et al.* (2013) targeted *Rhizoctonia* spp. in soil using a baiting method from arable fields across Europe in 2009/2010 season. Results from the UK (n = 32) showed *R. solani* AG 9 was the main *Rhizoctonia* spp. occurring in 34% of fields followed by AG 3 (TB) (16%), AG 2-1 (13%), AG 5 (9%), AG 11 (6%) and AG 8 (3%). Interestingly Goll *et al.* (2013) found no *R. cerealis,* suggesting the soil baiting method favoured other more competitive species. However, other binucleate *Rhizoctonia* spp. were identified; AG C and AG I were found in 13% of samples followed by AG E and AG K in 3% of samples.

The few studies investigating populations of *Rhizoctonia* spp. in UK arable fields suggest there is a lack of awareness of potential pathogenic species causing disease in wheat.

1.8 Control of rhizoctonia diseases in wheat

1.8.1 Cultural control

Cultural practices are an effective and sustainable way to control diseases caused by *Rhizoctonia* spp. in wheat and they should be the primary methods of disease control. A range of cultural methods have been identified to control diseases in wheat caused by *Rhizoctonia* spp.

Rhizoctonia root rot is favoured by direct drilling systems where no cultivation is performed. Rhizoctonia root rot has been noted as an important yield-limiting disease within these systems (Pumphrey *et al.,* 1987; Cook and Haglund, 1991). This is due to the lack of soil disturbance which allows proliferation of hyphal networks and also greater numbers of volunteer weeds that act as a host (MacNish, 1985; Pumphrey *et al.,* 1987; Smiley *et al.,* 1996). Therefore, ploughing or cultivation down to 8-10cm depth can be an effective cultural control option as it disturbs the hyphal network, fragmenting inoculum sources thus creating inoculum sources with less disease potential (MacNish, 1985; Paulitz *et al.,* 2002). However, the timely elimination of volunteer plants and grass weeds, which acts as a 'green bridge' carrying inoculum into the next crop, is seen as the most significant cultural control option regarding control of rhizoctonia root rot (Cook, 2001). Roget *et al.* (1987) demonstrated that increasing the time interval between an application of glyphosate and sowing from the standard 2-3 days until 2-3 weeks greatly reduced incidence of root rot caused by *R. solani.* This practice is now widely adopted in some areas where rhizoctonia root rot is a major issue (Cook, 2001). Due to the wide host range of the pathogen it is generally thought that rotations do not perform a major role in disease reduction (Cook *et al.,* 2002). However, studies have shown that rotations can influence *Rhizoctonia* spp. in soil and the severity of rhizoctonia diseases. Boine *et al.* (2014) conducted an inoculated field trial with the sugar beet pathogen AG 2-2IIIB and found that growing of a non-host (wheat) prior to the host (sugar beet) significantly reduced potential inoculum in the soil. However, rotation can increase the severity of disease. In field experiments in South Australia, rhizoctonia root rot was more severe after pasture than a legume or wheat (Roget, 1995). This shows rotation can influence *Rhizoctonia* biomass in soil and the severity of disease. There is also the potential to screen soils to

identify which *Rhizoctonia* spp. are present. This would allow growers to change cultivars or modify control measures (e.g. rotations) for specific AG's. A routine soil DNA-based testing service is already available for Australian grain growers to identify *R. solani* AG 8 but also a range of other soil borne pathogens (e.g. *Gaeumannomyces graminis* var. *tritici*) (Ophel-Keller *et al.,* 2008). That said there is evidence of a natural decline in rhizoctonia root rot. Decline in patch size and incidence of rhizoctonia root rot have been shown in wheat mono-cultures (MacNish, 1988, Roget, 1995). The build-up of antagonistic soil microbes (e.g. *Trichoderma* spp.) to *Rhizoctonia* spp. could explain the decline in wheat mono-cultures (Chel and Baker, 1980; Mghalu *et al.,* 2007). The application of fertiliser can also help reduce rhizoctonia root rot. Although, from the literature it would seem how the fertiliser is applied is vitally important. Topdressings where fertiliser is applied to the surface of the soil seem to have little impact on control of rhizoctonia root rot (Pumphrey *et al.,* 1987; MacNish, 1988). However, the placed of phosphorus fertiliser directly below the seed resulted in a yield advantage when wheat was under pressure from root diseases (e.g. rhizoctonia root rot) (Cook *et al.,* 2000).

The incidence of sharp eyespot is influenced by crop succession, cultivation, soil pH and texture, fertilisation and sowing date (Colbach *et al.,* 1997b). Research has demonstrated that ploughing increases the severity of sharp eyespot compared to minimum tillage (Prew *et al.,* 1995). The increased disease incidence following ploughing may be due to infected host material being returned to the surface (Prew *et al.,* 1995; Colbach *et al.,* 1997b). However, it may also be that ploughing creates a more favourable environment for the pathogen to grow by aerating and drying the top soil (Jordan and Hutcheon, 2003) or a combination of both. Timing of sowing has also shown to influence sharp eyespot occurrence. When early *vs.* late sowing dates have been compared sharp eyespot incidence was higher in early compared to late sowing crops (Clarkson and Cook, 1983; Prew *et al.,* 1995; Colbach *et al.,* 1997b; Cromey *et al.,* 2006). This is due to *R. cerealis* having a longer period of time to infect the host before the onset of winter. Colbach *et al.* (1997b) also demonstrated that when a non-host was grown prior to host sharp eyespot incidence was decreased due to the removal of host tissue thus reducing potential inoculum levels. The literature also suggests

that sharp eyespot is favoured by soils with a slightly acidic pH (Pitt, 1964; Parry, 1990). However, soil texture is widely regarded as the main soil related factor governing the incidence of sharp eyespot. In the UK, Pitt (1964) observed that 74% of sharp eyespot incidences occurred on light sandy soils. This is further supported by Daamen and Stol, (1990) who also found the disease to be favoured by sandy soils.

1.8.2 Biological control

Biological control agents while still limited in their commercial availability offer a potential future control of rhizoctonia diseases without harming the environment. Biocontrol agents can control the pathogen through antibiosis, siderophore production, induced resistance and competition (Kloepper *et al.*, 1980; Cardoso and Echandi, 1987; Harris *et al.*, 1997).

The main microorganisms of promise as bio-control agents against *Rhizoctonia* spp. are bacterial (e.g. *Pseudomonas* spp. and fungal (e.g. *Trichoderma* spp.) (Innocenti *et al.*, 2003; Bailey *et al.*, 2004; Mghalu *et al.*, 2007; Weller, 2007; Mavrodi *et al.*, 2012). *Pseudomonas* spp. can produce secondary metabolites, for example siderophores, which limit iron availability to pathogens, and antibiotics, which make them well suited as biocontrol agents (Weller, 2007). Mavrodi *et al.* (2012) found *Pseudomonas* spp. differed in their ability to inhibit growth of *R. solani* AG 8 *in vitro* and to reduce disease in wheat seedlings with some strains showing high suppression and others none. However, in field studies there was no correlation shown between *Pseudomonas* spp. and rhizoctonia root rot incidence from within 32 bare patches expressing root rot symptoms at a site in New South Wales, Australia (Donn *et al.*, 2014). There are many *Pseudomonas* spp. and it seems that some species offer potential biocontrol agents while others offer no control. A major disadvantage of *Pseudomonas* spp. is they do not produce resting spores which makes formulation as a commercial biocontrol agents problematic (Weller, 2007). However, agronomic practices such as straw mulching have been found to increase *Pseudomonas* spp. leading to a suppression of sharp eyespot (Chen *et al.*, 2010). This demonstrated that it may be possible to induce bio-control agents through agronomic practices.

Innocenti *et al.* (2003) found that wheat seed treated with *Trichoderma atroviride, T. harzianum* or *T. longibrachiatum* significantly recued the incidence of sharp eyespot over the infected controls. The authors suggested hyper-parasitism and non-volatile metabolites controlled *R. cerealis.* However, Bailey *et al.* (2004) found in microcosms experiments that applying *Trichoderma viride* as a bio-control agent to radish changed the epidemiological dynamics of *R. solani* AG 2-1. Results showed 91.7% disease control in first crops but this dropped to 64.8% in second crops even thou *Trichoderma viride* was re-introduced at sowing. This was part due to the saprophytic growth on root debris amplifying inoculum between crops resulting in higher primary infections in the second crops. This questions the long term role biocontrol agents. However, combining biocontrol agents and fungicides may give more consistent control than biocontrol agents alone. This would also reduce the amount of fungicide released into the environment and aid in potential resistance problems. *Pseudomonas fluorescens* combined with the fungicide pencycuron significantly reduced rhizoctonia root rot caused by *R. solani* AG 8 over either treatment alone in 3-4 week old wheat seedlings (Duffy, 2000). Similar findings were also shown when *Verticillium biguttatum* combined with flutolanil and pencycuron increased the control of black scurf in potatoes (van den Boogert and Luttikholt, 2004).

1.8.3 Bio-fumigants

Members of the *Brassicaceae* family contain glucosinolates, the precursors of isothiocyanates, which are known to have fungicidal activity and therefore have potential as soil bio fumigants. Yulianti *et al.* (2007) amended soil with *Diplotaxis tenuifolia* (perennial wall-rocket) or *Brassicaceae nigra* (black mustard) at low (1% w/w) and high (5% w/w) rates which were then inoculated with *R. solani* AG 2-1 and incubated for 6 months. After this time 12 OSR seedlings were sown into each pot. The results showed damping off occurred at the lower rate but at the higher rate none occurred. A later study showed that three soils amended with *B. rapa* (e.g. field mustard) and *B. napus* (rapeseed) and incubated for one week before cabbage seedlings were sown, effectively prevented damping off caused by *R. solani* AG 4 (Ascencion *et al.,* 2015). Disease control with the use of *Brassica* amendments has the potential to reduce

the use of artificial chemicals such as fungicides but needs further investigation before applying on a large scale. Factors such as the phytotoxicity to arrange of crops and the long term impact on soil microbial communities are not fully understood (Ascencion *et al.,* 2015). These studies suggest the long term implications of bio-fumigants needs to be further investigated.

1.8.4 Host resistance

Natural resistance expressed by the host is considered the most sustainable and environmental safe method of controlling diseases caused by *Rhizoctonia* spp. However, the level of tolerance expressed by different lines of wheat has been shown to vary and none have demonstrated true resistance (Neate, 1989; Smith *et al.,* 2003a; Smith *et al.,* 2003b; Oros *et al.,* 2013). Therefore, there are no wheat varieties completely resistant to rhizoctonia root rot currently available to growers. However, some mutant wheat lines have shown increased tolerance to *R. solani* AG 8 (Okubara *et al.,* 2009; Okubara & Jones, 2011). The mechanisms controlling resistance to *R. solani* AG 8 are mostly unknown. However, current work with *Brachypodium distachyon* may aid in the understanding of potential resistance mechanisms in wheat (Schneebeli *et al.,* 2015). Varieties that offer immunity or high resistance to sharp eyespot have also yet to be identified (Li *et al.,* 2011). This in part is down to the difficulty of breeding for sharp eyespot resistance in wheat using traditional methods something that may be overcome by genetic engineering (Chen *et al.,* 2008; Li *et al.,* 2011). However, in China quantitative resistance has been identified in two breeding lines. These were crossed and the resulting populations were found to have seven quantitative trait loci (QTL) associated with sharp eyespot resistance (Chen *et al.,* 2013).

1.8.5 Chemical control

As already mentioned wheat is susceptible to a range of *Rhizoctonia* spp. and is often attacked by multiple species and AG's during the crops development. Therefore, to control multiple infections by various *Rhizoctonia* spp. fungicides should, ideally, be active against all species and AG's (Kataria and Gisi, 1999). However, fungicides can show considerable variability in the control between

species, AG's and even isolates (Carling *et al.,* 1990; Kataria *et al.,* 1989; Olaya *et al.,* 1994; Smiley *et al.,* 1990; Campion *et al.,* 2003; Chen *et al.,* 2014; Zhang *et al.,* 2015). To control *Rhizoctonia* spp. fungicides can be applied as seed treatments, incorporated into soil or as foliar applications.

1.8.5.1 Seed treatments

Early control of *Rhizoctonia* spp. is critical as infection at the establishment phase of development can lead to plant death resulting in a substantial thinning out of stands. Therefore, seed treatments can offer vital protection during the period when plants are susceptible to attack. Ideally a seed treatment should be systemic, highly persistent and able to control all species and AG's (Kataria and Gisi, 1999; Hamada *et al.,* 2011a). A commonly used seed treatment is the phenylpyrrole fungicide fludioxonil which is known to have good activity to a range of multinucleate and binucleate *Rhizoctonia* spp. (Olaya *et al.,* 1994; Bains *et al.,* 2002; Hamada *et al.,* 2011b). Hamada *et al.* (2011) showed *R. cerealis* isolates (*n* =89) *in vitro* were most sensitive to fludioxonil (0.033 mg a.i ml^{-1}) but also to difenoconazole (0.062 mg a.i ml^{-1}) and iprodione 0.419 (mg a.i ml^{-1}). In an earlier *in vitro* study fludioxonil at 1 µg a.i ml^{-1} reduced hyphal growth of 98 multinucleate and nine binucleate *Rhizoctonia* isolates by an average of 99.5% (Oyala *et al.,* 1994). Fludioxonil has also shown good control in field studies. Baines *et al.* (2002) treated potato seed with six different fungicides and found the severity of stem canker and black scurf of potatoes was lowest in fludioxonil treated potatoes. In addition, the risk of resistance developing to fludioxonil is judged to be moderate; this is probably due to the mode of action of this fungicide (Xia *et al.,* 2012). Fungicides of the succinate dehydrogenase inhibitor (SDHI) group work by inhibiting the succinate dehydrogenase enzyme and therefore inhibit a fundamental step in the tricarboxylic acid cycle (TCA) and respiration process (Cecchini, 2003; Oyedotun and Lemire, 2004). Sedaxane is a SDHI fungicide that has shown good activity to a range of multinucleate and binucleate *Rhizoctonia* spp. (Zeun *et al.,* 2013; Goll *et al.,* 2014; Ajayi and Bradley, 2014). Goll *et al.* (2014) showed 105 *Rhizoctonia* isolates representing 16 AG's from soils across Europe were all sensitive to sedaxane with EC_{50} values between 0.001 and 0.093 mg a.i l^{-1}. Comparable EC_{50} values (≤0.012 mg a.i l^{-1})

were also found towards AG 1, 2-2IIIB, 3, 4, 5 and *R. cerealis* (Zeun *et al.*, 2013). The study by Zeun *et al.* (2013) also showed a yield advantage from sedaxane (65.62 dt ha^{-1}) treated wheat compared to the untreated (59.94 dt ha^{-1}) in field plots artificially inoculated with *R. cerealis.* The triazoles ipconazole and prothioconazole of the demethylation inhibitor class of fungicides are also commercially available seed treatments. There activity has, however, shown considerable variability with EC_{50} values for *R. solani* ranging from 0.21- 5.47 mg a.i l^{-1} and 0.20 to 5.58 mg a.i l^{-1} for ipconazole and prothioconazole respectively (Ajayi and Bradley, 2014). However, seed treatments are only considered effective at protecting seedlings in the early stages of infection (Paulitz *et al.,* 2002) offering only temporary defence until the roots have grown outside the zone of protection.

1.8.5.2 Foliar treatments

In wheat crops foliar treatments can be applied to control sharp eyespot. Foliar applications should be performed before stem extension (GS31) as the disease generally occurs before this time (Turner *et al.,* 2001; Nicholson *et al.,* 2002). However, chemical control of sharp eyespot is often variable (Parry, 1990; Ray *et al.,* 2004) and fungicides in the UK are rarely applied to target this disease as it is considered of low importance by growers and agronomists. Eyespot is considered the main stem base disease requiring specific fungicides to control at stem extension. There is evidence that fungicides applied to control eyespot can increase the incidence/severity of sharp which is likely to occur due to reduced competition (Prew and McIntosh. 1975; Van De Hoeven and Bollen, 1980; Clarkson and Cook, 1983). The active ingredient prochloraz is known to offer some suppression of the disease, but success is often inconsistent (Parry, 1990). In addition, the strobilurin fungicide, azoxystrobin has been shown to constantly decrease sharp eyespot and its pathogen *R. cerealis* (Bateman *et al.,* 2000) although the benefit of this fungicide used to directly control the disease is questionable (Bateman and Jenkyn, 2001). The succinate dehydrogenase inhibitor (SDHI) thifluzamide has also shown to be very effective against sharp eyespot in field studies in China (Sun *et al.,* 2015). However, this active is not currently registered for use in the UK.

1.9 Aims and objectives

The overall aim of this project was to elucidate crops losses and control of *R. cerealis* and *R. solani* in English wheat crops. The main objectives were to:

1) Investigate population dynamics of the main *Rhizoctonia* spp. and the principle pathogens causing the SBD complex in English winter wheat crops.

2) Determine the effects of meteorological variables and common agronomy practices on the main *Rhizoctonia* spp. and the principle pathogens causing the SBD complex in English winter wheat crops.

3) Determine the effects of disease caused by the main *Rhizoctonia* spp. on the biomass and yield of winter wheat and to evaluate the effectiveness of fungicide seed treatments as potential control options.

4) To characterise a range of multinucleate and binucleate *Rhizoctonia* spp. using ITS sequencing, pathogenicity and *in vitro* fungicide sensitivity experiments.

Chapter 2

General materials and methods

2.0 GENERAL METHODS

2.1 Storage and preservation of isolates of *Rhizoctonia* spp.

All isolates used in this study (appendix 10) were maintained on PDA slopes for short term storage or on barley grain at -25ºC for long term storage using a modified method by Sneh *et al*. (1991).

2.1.1 PDA slopes

PDA media (39g l^{-1} deionised water) was autoclaved at 121ºC for 15 minutes. The media was allowed to cool to 30-40ºC before pouring 30 ml into screw top Falcon® tubes (50 ml). Tubes were placed on an angle of 30-40º in a Laminar Flow Cabinet until media was set and all condensation had dried. One plug taken from the margin of an actively growing isolate on a PDA plate was then placed centre of the slope. The screw top was sealed with Parafilm® and slopes were stored at 15ºC in the dark.

2.1.2 Barley grain

Barley grain (250 g) was soaked overnight in water amended with 0.1 g l^{-1} of streptomycin and penicillin. Excess water was decanted and the grain autoclaved at 121ºC for 1 hr and left to cool at room temperature for 24 hr before autoclaving again at 121ºC for 1 hr. Once cooled, grain was then transferred to screw top glass vials (65 mm x 17 mm), which were filled approximately half full with caps loosely replaced before autoclaving a third time at 121ºC for 1 hr. The vials were then left to cool to room temperature. Plugs from the margin of actively growing cultures of 6-7 day old PDA plates were transferred to the vials which were then incubated at 20-23ºC for 7-10 days. Vials were then placed in a glass desiccator containing silica gel beads to remove moisture for 5-7 days or until dry. Tops of the vials were sealed with Parafilm M® tape and then placed in cold storage at -25ºC.

2.2 DNA extractions

2.2.1 Soil

Soil DNA was extracted throughout the period of this study using the method developed by Woodhall *et al.* (2012). In chapter 3 and 4 a standard soil sample size of 250 g was used. In chapter 5 the soil samples size was 50 g. Container size, CTAB volume, number of steal ball bearings and volume of Antifoam B for 50 g and 250 g samples are presented in Table 2.1. Soil samples were placed into the required sized Nalgene wide mouth environmental bottle with the necessary amount of steel ball bearings (25.4 mm), grinding buffer (120mM sodium phosphate buffer pH8, 2% cetrimonium bromide, 1.5 M sodium chloride) and Antifoam B. Nalgene bottles were then placed in a paint shaker (Merris Dispensing & Mixing Equipment, Czech Republic) and shaken for 4 minutes. A 50 ml sub-sample was then transferred to a 50 ml tube and centrifuged at 5000 g for five minutes. Twenty ml of the supernatant was added to a clean 50 ml tube containing 2 ml of 5M potassium acetate, tubes were then incubated on ice for 10 minutes. The tubes were then centrifuged at 12 000 g for 5 minutes. The supernatant was then added to a clean 50 ml tube containing 15 ml isopropanol and 800 µl 1% silicon dioxide suspension (Sigma). This was then placed on a flat-bed shaker for 15 minutes at 100 rpm followed by 5 minutes centrifugation at 12 000 g. The supernatant was discarded and 2 ml of Buffer A (Promega Wizard Food Kit) added to the remaining silicon dioxide particles. The tubes were then placed in a shaking incubator on their side for 10 minutes at 65°C at 100 rpm. The silica particles were then separated by centrifugation for 5 minutes at 12 000 g. Then 750 µl of the resulting supernatant was processed according to the manufacturer's instructions for the Wizard Food kit (Promega) in conjunction with a Kingfisher ML magnetic particle processor (Thermo Electron Corporation) using the genomic DNA (gDNA) programme with DNA eluted into 200 µl of TE buffer.

Table 2.1. Soil sample amount and related materials for DNA extractions

Sample amount (g)	Container size (ml)	CTAB Buffer amount (ml)	No. of steel balls	Antifoam (ml)
250	1000	500	20	15
50	250	100	6	3

2.2.2 Plant

2.2.2.1 CTAB

Unless otherwise stated this method was used to extract DNA from sampled wheat plants (e.g. roots or stem bases). Samples were freeze dried and then milled using a centrifugal mill (ZM 200, Retsch UK Ltd, Leeds) with a 1 mm screen. Between samples the mill was thoroughly cleaned to avoid cross contamination. Samples were stored at -20°C until DNA extractions were performed. DNA extraction followed the protocol described by Edwards *et al.* (2001). Four grams of milled sample was weighed into a 50ml falcon® tube with 30ml CTAB buffer (87.7 g sodium chloride, 25.0 g D-sorbitol, 10 g N-lauroylsarcosine sodium salt (Sarkosyl), 8.0 g N-hexadecyltrimethyl ammonium bromide (CTAB), 8.0 g Ethylenediamine tetraacetic acid (EDTA), 10 g Polyvinylpropylpyrrolidine (PVPP) with deionized water (DI) added to make a final volume of 1 litre). The content was mixed, and incubated at 65°C for 2 h in a water bath. The tubes were then cooled on ice and 10 ml of potassium acetate was added. Tubes were then inverted gently for 2-3 min followed by centrifugation for 15 minutes at 3000 g. From the resulting supernatant 1.2 ml was transferred into a 2 ml Eppendorf tube and 600 µl of chloroform was added. The content was mixed for 2 minutes and then centrifuged at 11 000 g for 15 minutes. One millilitre from the clear aqueous phase was then transferred into a 2 ml Eppendorf tube to which 800 µl of isopropanol (propan-2-ol 100%) was added and the contents mixed for 2 minutes before been placed at -20°C for 2 h to allow precipitation of DNA. Samples were centrifuged at 11 000 g for 15 minutes. The resulting pellet was washed twice with 1 ml isopropanol (44%) and then blotted to remove excess liquid and allowed to air dry overnight. Two hundred µl of Tris-EDTA (TE) buffer was added and tubes were place on a hot block for at 65°C for 2 hours, tubes were then vortexed and stored at -25ºC.

2.2.3 Fungi

2.2.3.1 *Rhizoctonia* spp.

Using a sterile scalpel approximately 200 mg of fungal mycelia was scraped from cultures grown on PDA for 6-10 days and placed in a 2ml screw cap microtube (Alpha laboratories, Eastleigh). One ml of buffer A (Promega Wizard Food Kit) was added to them and tubes were centrifuged for two 30 s cycles at 6000 rpm. One ml of the resulting supernatant was transferred to a clean 2 ml tube with 250 ml buffer B, and vortexed until turbid before adding 750 µl of precipitation solution. The mixture was centrifuged for 10 mins at 13000 g. Then 750 µl of the resulting supernatant was transferred to the first well of a kingfisher rack and processed according to the manufacturer's instructions for the Wizard Food kit (Promega) in conjunction with a Kingfisher ML magnetic particle processor (Thermo Electron Corporation) using the programme 'gDNA' (genomic DNA) with DNA eluted into 200 µl of TE buffer. Extracted fungal DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA). Isolates of known *Rhizoctonia* spp. which had been identified and verified by both morphological and molecular methods, maintained at Fera Science Ltd (Fera), Sandhutton, York, were used to make DNA standards for real time-PCR assays.

2.2.3.2 *Oculimacula* spp. and *Microdochium* spp.

Five plugs taken from 10-day old isolates grown on PDA were placed in 250 ml of potato dextrose broth (PDB) (Difco) and grown on a rotary shaker at 180 rpm for 7 days at 20 °C in the dark. After filtration and washing, the mycelium was freeze dried and ground with one sterile ceramic bead ball/sample in a 50 ml falcon® tube. Genomic DNA was extracted using the CTAB method described in section 2.2.2 with the following modifications; sample size was 0.5 g of ground mycelium and this was placed in 50ml falcon® tube and suspended in 9ml of CTAB buffer and thoroughly mixed. Tubes were placed in a water bath at 65^{o}C for two hours before cooling rapidly on ice before adding 3ml of potassium acetate. DNA quantification and dilution followed those described in section 2.2.4. Once diluted, a 10-fold serial dilution of the 1ng µl^{-1} sample was produced

to create samples with concentrations from 10^{0}-10^{-6} ng μl^{-1} which was used to make DNA standard curves in real time PCR assays.

Isolates of known *Oculimacula* spp. and *Microdochium* spp. which had been identified and verified by both morphological and molecular methods and maintained at University of Nottingham were used to make DNA standards.

2.2.4 DNA quantification

DNA in TE buffer was vortexed at 12 000 g for 5 minutes before making a 10 fold dilution which was centrifuged for 5 minutes at 12 000 g. DNA was quantified using a spectrophotometer (Varian Cary 50 UV-Vis). Prior to sample analysis the spectrophotometer was calibrated to zero using TE buffer. Fifty µl of diluted DNA extract was then placed in the cuvette and absorbance was measured at 260 nm, 280 nm, 328 nm and 360 nm wavelengths. DNA was quantified using Equation 1 where x = absorbance at 260 nm, y = absorbance at 328 and z = absorbance at 280nm:

$$\text{DNA concentration} = ((x - y) * 62.9) - ((z - y)*36)) \qquad \text{(Equation 1)}$$

All plant DNA was diluted to stocks of 20 ng μl^{-1} of total DNA in TE buffer prior to PCR analysis. Samples were then vortexed and centrifuged at 12 000 g for 5 minutes.

2.3 Real-time PCR

2.3.1 Quantification of *Rhizoctonia spp.*

The amplification and quantification of *Rhizoctonia* spp. in soil or plant material were performed using real-time PCR (TaqMan®) assays in 96 well plates using the Applied Biosystems 7500 real-time PCR system. Cycling conditions and reagents volumes are presented in Appendix 1. All *Rhizoctonia* spp. assays were performed at Fera, Sand Hutton, York. The following master-mixes were used for real-time PCR:

Environmental Master Mix 2.0 (Applied Biosystems) was used to target *Rhizoctonia* spp. in DNA extracts originating from soil samples. The Environmental Master Mix 2.0 consisted of half the total reaction volume of 25 µl with 5 µl consisting of DNA sample. Primers and probes (MWG Biotech, Germany) (Appendix 1) were added to a final concentration of 300 nM and 100 nM respectively with the remaining volume made up with molecular biology grade water.

Taqman® Gold (Applied Biosystems) was used to target *Rhizoctonia* spp. in DNA extract originating from plant material (e.g. roots and stems). The total reaction volume was 25 µl and consisted of 2.5 µl TaqMan® Buffer A, 5.5 µl Magnesium Chloride ($MgCl_2$), 2 µl Deoxynucleotide Triphosphates (DNTPs), 0.125 µl AmpliTaq Gold DNA Polymerase and 2 µl DNA sample. Primers and probes (MWG Biotech, Germany) (Appendix 1) were added to a final concentration of 300 nM and 100 nM respectively with the remaining volume made up with molecular biology grade water.

2.3.2 Analysis of real-time PCR (TaqMan®) assays

The cycle threshold (Ct) value for each reaction was assessed using the Sequence Detection Software's default threshold setting of 0.2 ΔRn (fluorescence) units. Each sample was tested in two replicates and an average Ct was taken. Primer and probe sequences for each *Rhizoctonia* spp. targeted are given in Appendix 1. Target DNA in soil samples was quantified by including five DNA standards on each PCR run. The standards consisted of a DNA sample of known concentration taken from the appropriate culture which was used to produce a dilution series of four ten-fold dilutions. Target DNA was then determined by linear regression.

2.3.3 Quantification of *Oculimacula* spp. and *Microdochium* spp.

The amplification and quantification of *Oculimacula* spp. and *Microdochium* spp. in soil or plant material was performed using real-time PCR assays in 96 well plates on the CFX96 Touch™ real-time PCR Detection System (Bio-Rad, UK).

Assays were performed at The University of Nottingham, Sutton Bonington campus. The total reaction volume for *O. yallundae* and *O. acuformis* was 25 µl comprising 5 µl DNA extract and 12.5 µl iQ SYBR® Green Supermix (Bio-Rad, UK). The final concentration of *O. acuformis* and *O. yallundae* primers and probes (Eurofins) were 300 nM (forward and reverse) and 100 nM respectively. Primer and probe sequences for *O. acuformis* and *O. yallundae* and cycle conditions are given in Appendix 1.

The total reaction volume for *M. nivale* and *M. majus* was 13 µl comprising of 2.5 µl DNA extract and 10.5 µl iQ SYBR® Green Supermix (Bio-Rad, UK). The final concentration of both *M. nivale* and *M. majus* primers (Eurofins) was 250 nM (forward and reverse). Primer sequences targeting the TEF-1α region of *M. nivale* and *M. majus* and cycle conditions are given in Appendix 1.

Pure DNA from known isolates of each species were included in each assay to make standard curves (10^{0} – 10^{-6} ng μl^{-1}) and target DNA was then quantified by linear regression. All real time PCR assays contained negative controls of nuclease free water.

Quantification of *Rhizoctonia* spp., *Oculimacula* spp. and *Microdochium* spp. DNA in soil samples was expressed as picograms of DNA per gram of soil (pg g^{-1} of soil) and for plant material as picograms per nanogram of total DNA (pg ng^{-1}).

2.4 Visual disease assessments

The following visual disease assessments keys were used throughout this study to assess for root rot, eyespot, brown foot rot (BFR) and sharp eyespot.

2.4.1 Root rot

Roots were visually assessed for root rot symptoms. Classification of root rot symptoms was previously described by Strausbaugh *et al.* (2004) (Appendix 2). Incidence was recorded as a percentage of the assessed plants showing symptoms of the disease. A disease index was calculated using equation 2 where, v = number of plants with <10% lesions on roots, w = number of plants with 10% - 33% lesions, x = number of plants with 34% - 66% lesions, y = number of plants with >66% lesions, z = total number of plants assessed.

Disease index (DI) = $((v + (w*2) + (x*3) + (y*4)) / (z*4)) * 100$ (Equation 2)

2.4.2 Eyespot, sharp eyespot and brown foot rot

Classification of eyespot, sharp eyespot and BFR severity was based on the key described by Scott and Hollins (1974) (Appendix 2).

Incidence was recorded as a percentage of the assessed plants showing symptoms of the disease. Disease index was calculated using equation 3, where v = number of plants with slight symptoms, w = numbers of plants with moderate symptoms, x = number of plants with severe symptoms and y = total number of plants assessed.

Disease index (DI) = $((v + (w*2) + (x*3)) / (y*3)) * 100$ (Equation 3)

Chapter 3

Population dynamics of *Rhizoctonia* spp., *Oculimacula* spp. and *Microdochium* spp. in English wheat crops

3.1 INTRODUCTION

This chapter describes the population dynamics of *Rhizoctonia* spp., *Oculimacula* spp. and *Microdochium* spp. in the soil and *in planta* of English wheat crops. In addition, disease affecting the roots (root rot) and stems (eyespot, BFR and sharp eyespot) are also described. Meteorological data for each site was collected to investigate correlations between pathogens or disease with temperature, rainfall, and humidity. The rationale behind this study is the limited information on the population dynamics of *Rhizoctonia* spp., *Oculimacula* spp. and *Microdochium* spp. in English wheat crops. Usually these pathogens are investigated in isolation although they often occur as complexes on roots and stem-bases and are therefore likely to interact and influence final disease occurrence and severity.

Stem base diseases of wheat commonly has three components, eyespot, brown foot rot (BFR) and sharp eyespot, which collectively are known as the stem base disease (SBD) complex (Nicholson and Turner, 2000). Eyespot is caused by either *Oculimacula acuformis* or *Oculimacula yallundae*; BFR is principally caused by *Microdochium nivale* or *Microdochium majus* and sharp eyespot is caused by *Rhizoctonia cerealis* (Polley and Thomas, 1991; Turner *et al.,* 2002; Crous *et al.,* 2003). However, the population dynamics of species in soil and *planta* are constantly changing due to selection pressure from environmental (e.g. meteorological variables) and agronomic (e.g. fungicides) factors. Changes in populations will impact on the effectiveness of control strategies. Therefore, current information on the predominant species is vital to help inform effective management decisions. Previous studies have found that *O. acuformis* is the main eyespot causing pathogen in the UK (Polley and Turner, 1995; Turner *et al*. ,1999; Nicholson and Turner, 2000). However its relative abundance declined between 1997 and 2000 due to changes in fungicide use such as the introduction of the fungicide cyprodinil which selects for *O. yallundae* (Parnell *et al*., 2008). The current dominant eyespot causing species is not known. The predominant *Microdochium* spp. in UK wheat is currently thought to be *M. majus* (Lees *et al.*,1995; Parry *et al*., 1995; Turner *et al.,* 1999) although seasonal variations are known to occur (Nicholson and Turner, 2000). In addition, the distribution, relative abundance and pathogenic activity of *R. cerealis* is thought to be

increasing due to climate change and changes in agronomic practices such as earlier sowing dates (Hamada *et al.*, 2011a).

There is also limited information on the population dynamics of other *Rhizoctonia* spp. in English wheat crops. *Rhizoctonia solani* is a soil-borne pathogen that causes disease in a broad range of crops including wheat (Mazzola *et al*., 1996; Ogoshi, 1996). *Rhizoctonia solani* is a species complex comprising 13 (1-13) anastomosis groups (AGs) (Carling *et al*., 2002a). Symptoms caused by these pathogens include pre- and post-emergence damping off, root rot, foliar blight and stem rots. *Rhizoctonia solani* AGs 2-1, 4, 5, 8 & 11 are also known to cause disease on the roots or stems of wheat (Ogoshi *et al*., 1990; MacNish & Neate, 1996; Demirci, 1998; Woodhall *et al*., 2012). Isolates of *R. solani* attack young roots of their hosts causing damage to the root system (Harris and Meon, 1985). For example, a recent study has shown that AG 2-1 is capable of reducing the number of primary roots, root volume and root surface area on six day old wheat seedlings (Sturrock *et al.*, 2015).

Environmental factors such as meteorological conditions are most likely to influence the development of fungal pathogens in soil and *in planta* and consequently alter root and stem base disease development and severity. Moisture is a key environmental factor required for eyespot infection to occur (Fitt *et al.*, 1988). Sporulation of ascospores and conidia is dependent on the moisture absorbed by infected straw on which the pathogen resides (Glynne, 1953; Rowe and Powelson, 1973b). Burpee *et al.* (1980b) showed that the disease index for wheat seedlings inoculated with *R. cerealis* declined as temperatures increased from 16°C to 28°C. Xu *et al.* (2008) over four years and four EU countries found that weather variables favouring concentrations of *M. nivale* and *M. majus* DNA were cooler temperatures and regular rainfall events.

The aim of this study was to investigate the population dynamics of the main *Rhizoctonia* spp. and the principle pathogens causing SBD complex in English winter wheat crops.

The individual objectives were to:

i) Determine the incidence and severity of diseases on the roots and stems of winter wheat crops.

ii) Quantify the population dynamics of the *Rhizoctonia* spp., *Oculimacula* spp., and *Microdochium* spp. in soil and *in planta* (e.g. roots and stems) at different developmental stages of the crop.

iii) Identify meteorological factors (e.g. rainfall and temperature) that correlate with disease severity and pathogen DNA concentrations in soil and *in planta*.

iv) Investigate associations at key crop developmental stages between disease, pathogen DNA concentrations in soil and *in planta*, and meteorological factors.

The specific null hypotheses tested within this chapter are:

i) There will be no difference in the incidence or severity of root rot and stem base diseases in winter wheat crops.

ii) The population dynamics of the *Rhizoctonia* spp., *Oculimacula* spp., and *Microdochium* spp. in soil and *in planta* will be the same.

iii) Meteorological factors will have no effect on diseases or pathogen DNA concentrations in soil and *in planta.*

iv) There will be no associations between disease, pathogen DNA and meteorological factors.

3.2 MATERIALS AND METHODS

3.2.1 Soil and plant sampling

The present study sampled 102 commercial winter wheat fields on 22 farms in 20 counties across England. Appendix 3 provides details of the location of farms and the numbers of fields sampled per farm. Farms were chosen to give a representative sample of winter wheat fields in the North, Midlands and South of England. On each farm the fields were randomly selected except for the criteria that wheat was the predominant crop within the field's rotation. This resulted in soil and plant samples being collected from a wide range of soil textures, environmental conditions and production systems that occur in England. During the 2011/12 and 2012/13 seasons, 52 and 50 fields were sampled, respectively. Farms sampled in 2011/12 were retained for the following season but new fields were randomly selected.

In each season, soil was sampled from each field were collected prior to the sowing of the crop (pre-sowing) and there after soil and plant samples were collected at GS 21-31, GS 37-45 and GS 65-75 (Zadoks *et al*., 1974). At each sampling period samples were removed from the same one ha area of the field, allowing changes in disease progression and pathogen populations to be observed in the same area. The area sampled was chosen away from the headland, in the mid-field area of the crop. Globally Positioning System (GPS) coordinates of the area sampled within each field were taken using Google Earth (Google Earth, 2011). This study used a systematic pattern of collecting soil and *planta* as this is provides a better estimate of changes of populations over random sampling methods (Campbell and Neher, 1994). Samples were removed along 5 x 100m parallel transects, spaced at 20m intervals. Along each transect six soil cores and six plants were removed, totalling 36 soil cores and 36 plants from each field. Soil cores were taken to a depth of 10-15 cm. Plants retaining all tillers were harvested along with the top 10-15 cm of the root system. Soil and plant material were placed in separate polythene bags and stored within 12 h of removal at 4-5°C for no longer than 36 h until disease assessments were performed. Once disease assessments were conducted plant material was frozen at -25°C until DNA extractions could be done.

3.2.2 Disease assessments

Roots were visually assessed for root rot symptoms and stems were visually assessed for eyespot, BFR and sharp eyespot symptoms. Stems were visually assessed for eyespot, sharp eyespot and brown foot rot at GS 21-31 and GS 37-45 on all tillers and at GS 65-75 on the main stem of each plant. All tillers were assessed at early growth stages to try and increase the detection of these diseases which can be difficult to identify early in the season. Refer to section 2.4 for full method details of the classification of root rot, eyespot, BFR and sharp eyespot symptoms.

3.2.3 DNA extractions

3.2.3.1 Soil

DNA was extracted from 250 g of soil throughout the period of this study using the method developed by Woodhall *et al.* (2012a). The 36 soil cores removed from each field were first homogenised to allow a representative sample to be extracted from. Refer to section 2.2.1 for full method details.

3.2.3.2 Plant

DNA from the plant samples was extracted from three fractions i) roots, ii) stem bases (5 cm of the stem basal region) at GS21-31, GS37-45 and GS65-75 and iii) stems (15 cm above the top 5 cm basal region) only at GS 37-45 and GS 65-75. Following disease assessments individual plant fractions (tissues) were finely chopped, homogenised and freeze dried. Refer to section 2.2.2 for full method details.

3.2.4 Real-time PCR assays

Real time PCR assays were performed on DNA extract from soil, roots, stem bases and stems. *Rhizoctonia* spp. targeted were *R. solani* AG 2-1, 3 (PT), 4 (I, II, II), 5, 8 and 9, *R. cerealis.* Real time PCR assays also targeted *O. yallundae*, *O. acuformis*, *M. nivale* and *M. majus.* Refer to section 2.3 for full method details. Table 3.1 presents which species were targeted in soil or plant fraction.

Table 3.1 Pathogens targeted in soil, root, stem bases or stems by real-time PCR assays

Pathogen	Soil	Roots	Stem bases[1]	Stems[2]
Rs AG 2-1	✓	✓	✓	×
Rs AG 3 (PT)*	✓	×	×	×
Rs AG 4-I*	✓	×	×	×
Rs AG 4-II*	✓	×	×	×
Rs AG 4-III*	✓	×	×	×
Rs AG 5+	✓	×	×	×
Rs AG 8+	✓	×	×	×
Rs AG 9*	✓	×	×	×
R. cerealis	✓	✓	✓	✓
O. yallundae	✓	✓	✓	✓
O. acuformis	✓	✓	✓	✓
M. nivale	✓	×	✓	×
M. majus	✓	×	✓	×

Rs – *Rhizoctonia solani*
✓ - Real-time assays were performed.
× - No real-time assays were performed.
[1] - Stem bases - 5 cm of the stem basal region.
[2] - Stems - 15 cm above the top 5 cm basal region.
* - Only PCR assays were performed on pre-sowing samples in 2011/12
+ - Only PCR assays were performed on pre-sowing samples in 2011/12 and 2012/13

3.2.5 Meteorological data

Meteorological records for mean daily maximum ($Temp_{max}$), minimum ($Temp_{min}$) temperature (°C), total rainfall (mm) and mean daily relative humidity (%) were obtained from the ECMWF (European Centre for Medium-Range Weather Forecasts). The ECMWF uses a numerical model system combined with observational data for reanalysis of the past weather. Therefore, the meteorological data presented are estimates based on the ECMWF model output for the time period of this study. The area the data is calculated covers a grid with a spatial resolution of 0.25 degree Latitude/Longitude (grid cell size ~27.5 kilometers). The latitude/longitude of each field was obtained using Globally Positioning System (GPS) coordinates of the area sampled within each field using Google Earth (Google Earth, 2011). Field coordinates were matched to the

nearest grid cell in the ECMWF model to obtain records for that particular field. Records of the meteorological factors for each field were compiled from the date the first samples were collected (pre-sowing) until the date the last samples were collected (GS65-75) in each survey. Table 3.7 presents the means for each meteorological variable. Data from the meteorological records were split into three intervals based on the period of time between sampling of each field. These intervals were linked to the seasons during the period of study. Therefore, the interval between pre-sowing to GS 21-31 is the autumn/winter period, the interval between GS 21-31 and GS 37-45 is spring and GS 37-45 to GS 65-75 is summer.

3.2.6 Statistical analysis

Genstat version 16.1 (VSN International Ltd., UK) was used for all statistical analysis. Quantified DNA of *Rhizoctonia* spp., *Oculimacula* spp., and *Microdochium* spp. is presented as means with 95% confidence intervals for each species in soil and *planta*. Correlations coefficients (r) were performed between diseases or pathogen DNA in soil or *planta* with their associated meteorological interval. For example, root rot at GS 21-31 was correlated with meteorological variables in the autumn/winter period (pre-sowing and GS21-31) and root rot at GS 37-45 was correlated with meteorological variables in the spring (GS21-31 to GS 37-45). Only significant correlations at $P \leq 0.05$ presented. Further associations were explored between diseases, pathogens and meteorological variables at each sampling period in the soil, roots, stem bases and stems using principle components analysis (PCA). The results of the PCA analysis are graphically drawn as biplots, showing the variables as vectors. Vectors that follow acute angles are associated while vectors that are perpendicular have no association and vectors in opposing directions have negative associations. DNA data was $\log_{10}$ transformed to normalise residual distribution before correlations coefficients and PCA analysis were performed.

3.3 RESULTS

3.3.1 Incidence and severity of root rot and stem base diseases

Disease incidence (I) as a percentage of fields (F) sampled and as a percentage of plants (P) sampled per field and disease index (DI) for brown foot rot, eyespot, sharp eyespot and root rot are shown in Table 3.2. Disease incidence as a percentage of fields sampled showed root rot was present in >90% of crops at GS 21-31 but showed a declining trend over both seasons. BFR incidence varied between 92-100% of crops and was the dominant SBD throughout the period of study. Eyespot was more prevalent in 2011/12 than in 2012/13 occurring 100% and 98% of crops at GS 37-45 and GS 65-75 respectively. Sharp eyespot also occurred more frequently in 2011/12 than 2012/13 and was identified in 87 and 90% of crops at GS 37-45 and GS 65-75 respectively.

Disease incidences as a percentage of plants sampled per field identified root rot on ~27% of plants at GS 21-31 then again showed a declining trend over both seasons (Table 3.2). Overall BFR was the most common occurring SBD found on 24-46% of plants at each growth stage. Eyespot was highly prevalent in 2011/12 occurring on 61 and 49% of plants at GS 37-45 and GS 65-75 respectively. In contrast, eyespot in 2012/13 was identified at a relatively low incidence (<20%). Sharp eyespot incidence on plants was also higher in 2011/12 than 2012/13 with 20 and 26% of plants infected at GS 37-45 and GS 65-75 respectively.

Disease index is a measure of disease intensity (calculated from incidence and severity values). Root rot DI was relatively high at GS 21-31 (~14%) in both seasons but then declined by GS 65-75 (~4%) (Table 3.2). BFR generally had the highest DI of the SBDs (range14.1-30.7%). Eyespot DI in 2011/12 increased considerably between GS 21-31 (8.4%) and GS 37-45 (40.8%) but then declined at GS 65-75 (29.2%). In 2012/13 eyespot DI remained low (<12%) at each growth stage. Sharp eyespot had the lowest DI of the SBDs. In 2011/12 sharp eyespot DI increased from GS 21-31 (1.1%) to GS 65-75 (18.1%) but in 2012/13 remained low throughout the season (<7.5%).

Table 3.2 Mean (%) disease incidence (I) and disease index (DI) for root rot, eyespot, brown foot rot, sharp eyespot on English winter wheat plants collected at GS21-31, GS 37-45 and GS65-75 in 2011/12 and 2012/13

		GS 21-31		GS 37-45		GS 65-75	
		2011/12[a]	2012/13[b]	2011/12	2012/13	2011/12	2012/13
Root rot	I (F)[1]	100	94.0	94.0	82.0	52.0	58.0
	I (P)[2]	28.0	26.0	24.0	14.0	7.0	8.0
	DI	14.6	13.0	11.8	6.8	3.7	4.2
Eyespot	I (F)[1]	62.0	30	100	88.0	98.0	74.0
	I (P)[2]	13.0	3.0	61.0	16.0	49.0	15.0
	DI	8.4	2.2	40.8	10.8	29.2	11.7
BFR	I (F)[1]	92.0	94.0	100	100	100	100
	I (P)[2]	24.0	39.0	37.0	46.0	41.0	46.0
	DI	14.1	23.7	27.8	30.7	30.2	28.3
SE	I (F)[1]	17.0	50.0	87.0	64.0	90.0	52.0
	I (P)[2]	2.0	8.0.	20.0	10.0	26.0	6.0
	DI	1.1	4.9	13.4	7.1	18.1	5.0

[1] - Percentage of fields scored positive for disease. [2] - Percentage of plants scored positive for disease. [a] - 2011/12 n = 52 (total 1872 plants disease assessed per GS). [b] - 2012/13 n = 50 (total 1800 plants disease assessed per GS). sharp eyespot (SE), brown foot rot (BFR).

3.3.2 Pathogen presence and DNA concentrations in soil

Incidence and DNA concentrations of *Rhizoctonia* spp., *Oculimacula* spp., and *Microdochium* spp. in soil samples collected from winter wheat crops are presented in Table 3.3.

Throughout the period of study the most frequent species detected in soil samples was *O. acuformis* (73%). This was followed by *O. yallundae* (68%), *R. solani* AG 2-1 (63%), *R. cerealis* (54%), *M. nivale* (38%) and *M. majus* (28%). The incidence of *R. solani* AG 2-1, *R. cerealis* and *O. acuformis* remained relatively constant between seasons and sampling periods. *Oculimacula yallundae* incidence showed a declining trend in 2011/12 from pre-sowing (90%) to GS 65-75 (64%); this trend was not shown in 2012/13 (range 51-68%). The occurrence of *M. nivale* DNA in soil samples in both seasons increased >100% from pre-sowing to GS 21-31. In 2011/12 *M. majus* frequency was low throughout the period of study, occurring in ≤20% of samples, while in the following season incidence increased from pre-sowing (33%) to GS65-75 (62%).

In terms of DNA concentrations *R. solani* AG 2-1 was the predominant species detected in soil samples throughout the period of study (Table 3.3). This was followed by *O. yallundae, R. cerealis*, *O. acuformis, M. nivale*, and *M. majus*. The amount of pathogen DNA in soil samples showed changes between sampling periods and seasons. *R. solani* AG 2-1 DNA levels were higher in 2011/12 than 2012/13 at each sampling period. The overall trend in both seasons showed AG 2-1 DNA generally declined between sampling periods. *R. cerealis* DNA in soil was also higher in 2011/12 than 2012/13. In both seasons *R. cerealis* DNA levels in soil showed the same trend, increasing over the autumn/winter period then decreasing in the spring (GS 37-45) before increasing in the summer (GS 65-75). The DNA concentrations of *O. yallundae* in soil samples were higher than *O. acuformis* throughout the period of study. There was no overall pattern of development of *O. yallundae* DNA in soil. *O. acuformis* DNA was quantified at considerably higher amounts in 2012/13 than in 2011/12 at each sampling period but over the season showed a declining trend. In 2011/12 *M. nivale* DNA levels in soil were higher than *M. majus* but the reverse was shown in 2012/13. The largest change in *Microdochium* spp. DNA concentrations occurred over the autumn/winter in 2011/12 when *M. nivale* DNA increased 46 fold.

This study also detected AG 5 (5%) and AG 8 (2%) in pre-sowing soil samples in 2011/12. In pre-sowing samples in 2012/13 AG 8 was again detected in 2% of soil samples. *R. solani* AG 5 DNA concentrations ranged was 2.12-12.22 pg g^{-1} of soil and AG 8 was 4.71-6.81 pg g^{-1} of soil. Due to the low incidence no further PCR assays were performed on these species. In addition, no AG 3 (PT), AG 4 (I, II, III) or AG 9 was detected in pre-sowing samples in 2011/12 (data not shown). Again due to the low incidence no further PCR assays were performed on these species.

Table 3.3 Pathogen DNA in soil samples from English winter wheat fields collected at pre-sowing , GS 21-31, GS 37-45 and GS 65-75 in 2011/12 and 2012/13 seasons

	Fungal DNA (pg g^{-1} of soil) mean ± 95% confidence interval (incidence %)							
	Pre-sowing		**GS21-31**		**GS37-45**		**GS 65-75**	
	2011/12[a]	**2012/13[b]**	**2011/12[c]**	**2012/13[d]**	**2011/12**	**2012/13**	**2011/12**	**2012/13**
AG2-1	427.3 ± 326.1 (67)	376.6 ± 245.9 (67)	943.0 ± 685.3 (56)	200.3 ± 124.9 (64)	404.6 ± 378.3 (58)	230.9 ± 151.52 (67)	187.8 ± 102.9 (62)	125.4 ± 97.23 (65)
Rc	103.2 ± 50.45 (62)	30.50 ± 23.90 (50)	139.8 ± 80.61 (58)	89.24 ± 47.10 (54)	73.16 ± 44.63 (52)	22.74 ± 14.29 (44)	130.14 ± 73.38 (60)	53.28 ± 37.77 (54)
OA	2.83 ± 2.38 (67)	28.66 ± 44.54 (67)	0.76 ± 0.38 (81)	14.62 ± 16.49 (74)	0.39 ± 0.28 (69)	23.53 ± 30.90 (71)	0.55 ± 0.26 (80)	11.57 ± 12.21 (80)
OY	32.90 ± 31.97 (90)	135.9 ± 140.9 (51)	215.9 ± 406.2 (75)	85.93 ± 158.8 (68)	41.61 ± 64.08 (63)	33.83 ± 41.67 (67)	18.20 ± 16.20 (64)	126.8 ± 239.6 (68)
Mn	0.28 ± 0.29 (10)	4.94 ± 4.56 (22)	12.87 ± 10.00 (62)	1.96 ± 0.99 (45)	2.47 ± 2.68 (29)	3.45 ± 1.66 (44)	4.32 ± 2.38 (44)	3.87 ± 2.60 (44)
Mm	0.18 ± 0.18 (10)	5.24 ± 4.13 (33)	0.07 ± 0.10 (4)	2.32 ± 1.14 (45)	0.61 ± 0.87 (4)	2.08 ± 1.04 (48)	1.23 ± 1.57 (20)	8.87 ± 3.35 (62)

R. solani (AG2-1) and *R. cerealis* (Rc), *O. acuformis* (OA), *O. yallundae* (OY), *M. nivale* (Mn), *M. majus* (Mm). [a] - Pre-sowing 2011/12 n = 42. [b] - Pre-sowing 2012/13 n = 46. [c] - 2011/12 (GS 21-31 onwards) n = 52. [d] - 2012/13 (GS 21-31 onwards) n = 50.

3.3.3 Pathogen presence and DNA concentrations in roots

On average *O. yallundae* (90%) and *O. acuformis* (88%) were the most frequent species detected in root samples (Table 3.4). These were followed by *R. cerealis* (56%) and AG 2-1 (52%). The species with the highest DNA concentrations on the roots of wheat was *O. yallundae.* This was followed by *O. acuformis, R. cerealis* and, at relatively trace amounts AG 2-1. DNA of each species increased from GS 21-31 to GS 65-75 in both seasons. There were notably high concentrations of *O. yallundae* DNA detected at GS 65-75 in 2011/12 (1.37 pg ng^{-1} of total DNA) and in 2012/13 (8.01 pg ng^{-1}).

Table 3.4 Pathogen DNA in roots of English winter wheat plants collected at GS21-31, GS 37-45 and GS65-75 in 2011/12 and 2012/13

	Fungal DNA (pg ng^{-1} of total DNA) mean ± 95% confidence interval (incidence %)					
	GS21-31		**GS37-45**		**GS65-75**	
	2011/12[a]	**2012/13[b]**	**2011/12**	**2012/13**	**2011/12**	**2012/13**
AG2-1	0.001 ± 0.001 (48)	0.00001 ± 0.00001 (11)	0.0002 ± 0.0002 (50)	0.001 ± 0.001 (71)	0.004 ± 0.006 (69)	0.001 ± 0.001 (62)
Rc	0.07 ± 0.03 (69)	0.06 ± 0.05 (44)	0.32 ± 0.16 (69)	0.04 ± 0.03 (41)	0.16 ± 0.06 (73)	0.14 ± 0.09 (40)
OA	0.01 ± 0.01 (60)	0.03 ± 0.01 (96)	0.28 ± 0.20 (90)	0.06 ± 0.03 (90)	0.31 ± 0.14 (96)	0.29 ± 0.23 (98)
OY	0.01 ± 0.01 (67)	0.03 ± 0.02 (93)	1.27 ± 0.74 (98)	0.21 ± 0.19 (88)	1.37 ± 0.52 (94)	8.01 ± 4.71 (98)

R. solani (AG2-1) and *R. cerealis* (Rc), *O. acuformis* (OA), *O. yallundae* (OY). [a] - 2011/12 $n = 52$. [b] - 2012/13 $n = 50$

3.3.4 Pathogen presence and DNA concentrations in stem bases

Incidence and DNA concentrations of *Rhizoctonia* spp., *Oculimacula* spp., and *Microdochium* spp. in stem bases of winter wheat are presented in Table 3.5. *Oculimacula* spp. and *Microdochium* spp. were the most frequently detected species, present in >90% of samples at each growth stage. Less frequently found were *R. cerealis* (74%) and AG 2-1 (39%). The incidence of *R. cerealis* was, on average, higher in 2011/12 (95%) samples than 2012/13 (53%). *Rhizoctonia solani* AG 2-1 incidence varied between 13% and 64% of samples throughout the period of study.

In both seasons *O. yallundae* was the predominant species in stem bases followed by *O. acuformis, M. nivale, M. majus,* and *R. cerealis* (Table 3.5). Only trace amounts of AG 2-1 were quantified in stem bases. There were changes in the amount of pathogen DNA in stem bases between growth stages and seasons. In both seasons the highest amount of pathogen DNA at GS 21-31 were those of *M. nivale.* In 2011/12 *M. nivale* DNA declined 19 fold between GS 21-31 and GS 37-45 but in the following season increased at each successive growth stage. DNA of the *Oculimacula* spp. showed an increasing trend between growth stages in both seasons. The largest increase for both species occurred between GS 37-45 and GS 65-75 in 2012/13 when DNA increased 27 and 52 fold for *O. acuformis* and *O. yallundae* respectively. *Rhizoctonia cerealis* DNA in stem bases was relatively high at GS 21-31 in 2011/12 but failed to develop over the season. In contrast *R. cerealis* showed an increasing trend between growth stages in the following season. DNA concentrations of *M. majus* in stem bases generally showed an increasing trend between growth stages in both seasons.

Table 3.5 Pathogen DNA in stem bases (5 cm stem basal region) of English winter wheat plants collected at GS21-31, GS37-45 and GS 65-75 in 2011/12 and 2012/13

	Fungal DNA (pg ng^{-1} of total DNA) mean ± 95% confidence interval (incidence %)					
	GS 21-31		**GS 37-45**		**GS 65-75**	
	2011/12[a]	**2012/13[b]**	**2011/12**	**2012/13**	**2011/12**	**2012/13**
AG2-1	0.0004 ± 0.001 (43)	0 .00002 ± 0.00002 (13)	0.0001 ± 0.0001 (21)	0.00008 ± 0.00004 (40)	0.001 ± 0.0004 (64)	0.001 ± 0.001 (51)
Rc	1.83 ± 0.70 (96)	0.09 ± 0.07 (50)	2.31 ± 0.94 (96)	0.16 ± 0.10 (48)	1.46 ± 0.62 (94)	1.19 ± 0.92 (61)
OA	0.23 ± 0.12 (100)	0.04 ± 0.02 (94)	1.85 ± 1.53 (100)	0.17 ± 0.15 (96)	9.70 ± 5.07 (100)	4.59 ± 2.91 (98)
OY	0.87 ± 0.52 (100)	0.10 ± 0.06 (94)	7.64 ± 3.64 (100)	2.14 ± 1.76 (96)	45.69 ± 14.27 (100)	110.9 ± 48.00 (98)
Mn	7.17 ± 1.50 (96)	0.50 ± 0.27 (97)	0.37 ± 0.20 (96)	0.70 ± 0.22 (96)	1.88 ± 1.26 (100)	3.15 ± 1.15 (92)
Mm	1.30 ± 0.50 (92)	0.17 ± 0.09 (90)	1.02 ± 0.43 (92)	1.23 ± 1.27 (98)	3.56 ± 3.22 (100)	2.59 ± 1.24 (100)

R. solani (AG2-1) and *R. cerealis* (Rc), *O. acuformis* (OA), *O. yallundae* (OY), *M. nivale* (Mn), *M. majus* (Mm). [a] - 2011/12 *n* = 52
[b] - 2012/13 *n* = 50

3.3.5 Pathogen presence and DNA concentrations in stems

Oculimacula spp. and *R. cerealis* were widespread on the stems of wheat at GS 37-45 and GS 65-75 generally occurring in >75% of samples (Table 3.6). However, *R. cerealis* in 2012/13 was rarely detected at GS 37-45 yet by GS 65-75 was present in 78% of samples. Overall the dominant species in terms of DNA was *R. cerealis* (Table 3.6). In both season the amount of *R. cerealis* DNA increased in stem samples, most notably in 2012/13 when DNA increased >1500 fold. DNA concentrations of *O. acuformis* on stems increased 32 fold in 2011/12 but in the following season failed to develop. The amount of *O. yallundae* DNA on stems increased in both season, this was most notable in 2011/12 when the pathogen increased 10 fold.

Table 3.6 Pathogen DNA in stems (15 cm above the 5 cm basal region) of English winter wheat plants collected at GS37-45 and GS 65-75 in 2011/12 and 2012/13

	Fungal DNA (pg ng^{-1} of total DNA) mean ± 95% confidence interval (incidence %)			
	GS 37-45		**GS 65-75**	
	2011/12[a]	**2012/13[b]**	**2011/12**	**2012/13**
Rc	1.67 ± 0.85 (89)	0.004 ± 0.005 (10)	4.90 ± 2.03 (90)	6.74 ± 5.60 (78)
OA	0.14 ± 0.09 (89)	0.91 ± 1.76 (80)	4.45 ± 3.19 (98)	0.41 ± 0.57 (76)
OY	0.33 ± 0.20 (94)	1.55 ± 2.52 (76)	3.44 ± 2.11 (98)	2.33 ± 2.08 (98)

R. cerealis (Rc), *O. acuformis* (OA) and *O. yallundae* (OY). [a] - 2011/12 n = 52, [b] - 2012/13 n = 50.

3.3.6 Meteorological data for English wheat fields sampled in 2011/12 and 2012/13

Meteorological data was split into three intervals based on the period of time between sampling of each field. These intervals were linked to the seasons during the period of study. Therefore, the interval between pre-sowing to GS 21-31 is the autumn/winter period, the interval between GS 21-31 and GS 37-45 was the spring period and GS 37-45 to GS 65-75 was the summer period.

Meteorological data showed that throughout 2011/12 the mean $Temp_{max}$ and $Temp_{min}$ were higher than in 2012/13 (Table 3.7). Mean total rainfall in 2012/13 over the autumn/winter period was double that in 2011/12. In contrast, in 2011/12 rainfall in the spring period was slightly higher than in 2012/13. However, there was a considerable difference in rainfall in the summer period; in 2011/12 there was 146 mm more rainfall than in 2012/13. Mean relative humidity over the autumn/winter period was slightly higher in 2012/13 than 2011/12. However, in 2011/12 from GS 37-45 to GS 65-75 relative humidity was 3.4% and 4.7% higher in spring and summer than at the same periods in 2012/13.

Table 3.7 Mean temperature, relative humidity and total rainfall for fields sampled from pre-sowing to GS 65-75 across England

		Autumn/winter*	Spring	Summer
$Temp_{max}$ (^{o}C)	2011/12	10.4	13.0	18.2
	2012/13	8.7	11.1	20.4
$Temp_{min}$ (^{o}C)	2011/12	4.6	4.5	10.6
	2012/13	3.4	3.1	10.5
Total rain (mm)	2011/12	200	163	180
	2012/13	443	116	34
Relative humidity (%)	2011/12	82.5	78.6	80
	2012/13	84.8	75.2	75.3

* Autumn/winter - Pre-sowing to GS 21-31. Spring - GS21-31 to GS 37-45. Summer- GS37-45 to GS 65-75.

3.3.7 Correlation coefficients (*r*) between root rot and SBDs with meteorological variables

Root rot at GS 37-45 was positively correlated with each meteorological variable in the spring (Table 3.8). The strongest correlation was with relative humidity ($r = 0.50$).

Eyespot index at GS 21-31 was negatively associated with rainfall over the autumn/winter period (Table 3.8). Eyespot at GS 37-45 had strong positive correlations ($r > 0.55$) with each meteorological variable in the spring. However, eyespot at GS 65-75 was positively correlated with rainfall and relative humidity but negatively associated with $Temp_{max}$ in the summer period.

BFR was only correlated with variables in the autumn/winter period. There were positive correlations with rainfall and humidity and negative associations with $temp_{max\ \&\ min}$ (Table 3.8). The strongest of these correlations was with rainfall ($r = 0.53$)

Sharp eyespot at GS 21-31 had a weak but positive correlation with rainfall over the autumn/winter period (Table 3.8). In the summer period sharp eyespot had strong correlations with rainfall ($r = 0.60$), relative humidity ($r = 0.47$) and $temp_{max}$ ($r = -0.50$).

Table 3.8 Correlation coefficients (*r*) and probabilities (in parentheses) if $P \leq 0.05$ between disease index on roots and stems assessed at GS21-31, GS37-45 and GS65-75 in 2011/12 to 2012/13 and temperature (*max* and *min*), total rainfall and relative humidity in English winter wheat fields

	Root rot	**Eyespot**	**BFR**	**SE**
***Temp*$_{max}$ (°C)**[1]				
Autumn/winter[a]	ns	0.37 (<0.001)	-0.44 (<0.001)	ns
Spring[b]	0.34 (<0.001)	0.59 (<0.001)	ns	0.31 (0.0015)
Summer[c]	ns	-0.49 (<0.001)	ns	-0.50 (<0.001)
***Temp*$_{min}$ (°C)**[2]				
Autumn/winter	ns	0.33 (<0.001)	-0.41 (<0.001)	ns
Spring	0.38 (<0.001)	0.60 (<0.001)	ns	0.40 (<0.001)
Summer	ns	ns	ns	ns
Total rainfall (mm)				
Autumn/winter	ns	-0.33 (<0.001)	0.53 (<0.001)	0.24 (0.0176)
Spring	0.35 (<0.001)	0.59 (<0.001)	ns	0.29 (0.0031)
Summer	ns	0.57 (<0.001)	ns	0.60 (<0.001)
Relative humidity (%)				
Autumn/winter	-0.25 (0.0109)	-0.22 (0.0265)	0.26 (0.0093)	0.21 (0.0325)
Spring	0.50 (<0.001)	0.59 (<0.001)	ns	0.26 (0.0086)
Summer	ns	0.38 (<0.001)	ns	0.47 (<0.001)

Sharp eyespot (SE) and brown foot rot (BFR). ns = not significant ($P > 0.05$). [1] - *Temp*$_{max}$ - temperature maximum (°C). [2] - *Temp*$_{min}$ - temperature minimum (°C). [a] - Autumn/winter - Pre-sowing to GS 21-31. [b] - Spring - GS21-31 to GS 37-45. [c] - Summer- GS37-45 to GS 65-75.

3.3.8 Correlation coefficients (*r*) between pathogen DNA in soil and *planta* correlated with and meteorological variables

3.3.8.1 Soil

In soil samples *R. solani* AG 2-1 and *R. cerealis* DNA had no or weak correlations with the meteorological variables in each period (Table 3.9).

Oculimacula spp. DNA in soil had weak associations ($r \leq 0.30$) with meteorological variables in this study (Table 3.9). The strongest of these was a negative correlation with rainfall over the autumn/winter period ($r = -0.30$).

Microdochium nivale DNA had weak correlations with meteorological variables, the strongest was with rainfall ($r = -0.30$) over the autumn/winter period (Table 3.9).

Microdochium majus DNA at GS 21-31 was positively correlated with rainfall and negatively associated with $temp_{max\&min}$ over the autumn/winter period (Table 3.9). In the spring *M. majus* was negatively associated with each meteorological variable. However, in the summer *M. majus* was strongly ($r = 0.52$) correlated with $temp_{max}$ but had negative correlations with rainfall and relative humidity.

3.3.8.2 Roots

Rhizoctonia solani AG 2-1 DNA was again not correlated with any meteorological variable (Table 3.10). *Rhizoctonia cerealis* DNA in roots had weak correlations with meteorological variables ($r = <0.40$). The strongest correlation occurred with $temp_{min}$ ($r = 0.36$) in the spring period.

Oculimacula acuformis DNA in roots was also weakly correlated with meteorological variables (Table 3.10). The strongest but negative association was with $temp_{max\&min}$ over the autumn/winter period. DNA of *O. yallundae* had a relatively strong correlation at GS 37-45 with $temp_{max}$ ($r = 0.44$) in the spring.

3.3.8.3 Stem bases

In stem bases *R. solani* AG 2-1 DNA showed no association with any of the meteorological variables (Table 3.11). DNA of *R. cerealis* at GS 21-31 was correlated with $temp_{max\ \&\ min}$ (positive) and rainfall and relative humidity (negative) over the autumn/winter period. In the spring *R. cerealis* was positively associated with each variable; the strongest was with $temp_{max\ \&\ min}$ (r >0.50). In contrast *R. cerealis* DNA at GS 65-75 was negatively correlated with $temp_{max}$ but again positively associated with rainfall and relative humidity in the summer period.

Oculimacula spp. DNA in stem bases at GS 21-31 had strong negative correlations with rainfall and positive associations with $temp_{max\&min}$ over the autumn/winter period (Table 3.11). DNA of *Oculimacula* spp. showed weak (r <-0.35) positive association with rainfall and humidity in the spring. In the summer *O. acuformis* was negatively associated with $temp_{max}$ and positively correlated with rainfall and relative humidity; these associations were not shown for *O. yallundae.*

Microdochium nivale and *M. majus* DNA at GS 21-31 were positively correlated with $temp_{max\&min}$ and negatively associated with rainfall and humidity over the autumn/winter period (Table 3.11). There were no further significant correlations for the *Microdochium* spp. in the spring or summer periods.

3.3.8.4 Stems

In stems *R. cerealis* DNA at GS 37-45 showed strong positive correlations (r >0.60) with rainfall and $temp_{max\ \&\ min}$ and to a lesser extent relative humidity in the spring period (Table 3.12).

The strongest correlations for *O. acuformis* (r = 0.52) and *O. yallundae* (r = 0.39) DNA at GS 65-75 was with rainfall in the summer period (Table 3.12).

Table 3.9 Correlation coefficients (*r*) and probabilities (in parentheses) between pathogen DNA concentrations (log pg g^{-1} of soil) in soil samples collected at GS21-31, GS37-45 and GS65-75 in 2011/12 to 2012/13 temperature (*max* and *min*), total rainfall and relative humidity in English winter wheat fields

	AG2-1	**Rc**	**OA**	**OY**	**Mn**	**Mm**
***Temp*$_{max}$ (°C)[1]**						
Autumn/winter[a]	ns	-0.22 (0.0248)	-0.23 (0.0221)	ns	ns	-0.47 (<0.001)
Spring[b]	ns	ns	ns	ns	ns	-0.22 (0.0308)
Summer[c]	ns	ns	0.24 (0.0138)	ns	ns	0.52 (<0.001)
***Temp*$_{min}$ (°C)[2]**						
Autumn/winter	ns	ns	-0.24 (0.0156)	-0.23 (0.0244)	ns	-0.42 (<0.001)
Spring	ns	ns	ns	ns	ns	-0.26 (0.0095)
Summer	ns	0.32 (0.0012)	0.25 (0.0126)	ns	0.21 (0.0404)	ns
Total rainfall (mm)						
Autumn/winter	ns	ns	-0.30 (0.0024)	-0.29 (0.0035)	-0.30 (0.0022)	0.38 (<0.001)
Spring	ns	ns	ns	ns	ns	-0.39 (<0.001)
Summer	ns	ns	ns	ns	ns	-0.44 (<0.001)
Relative humidity (%)						
Autumn/winter	ns	ns	ns	-0.20 (0.0439)	-0.21 (0.0385)	ns
Spring	ns	ns	-0.26 (0.0082)	ns	ns	-0.33 (<0.001)
Summer	ns	ns	-0.24 (0.014)	ns	ns	-0.36 (<0.001)

R. solani (AG2-1) and *R. cerealis* (Rc), *O. acuformis* (OA), *O. yallundae* (OY), *M. nivale* (Mn), *M. majus* (Mm). ns = not significant (P > 0.05). [1] - *Temp*$_{max}$ - temperature maximum (°C). [2] - *Temp*$_{min}$ - temperature minimum (°C). [a] - Autumn/winter - Pre-sowing to GS 21-31. [b] - Spring 2 - GS21-31 to GS 37-45. [c] - Summer- GS37-45 to GS 65-75.

Table 3.10 Correlation coefficients (*r*) and probabilities (in parentheses) between pathogen DNA concentrations (log pg ng^{-1} of total DNA) in roots collected at GS21-31, GS37-45 and GS65-75 in 2011/12 to 2012/13 and temperature (*max* and *min*), total rainfall and relative humidity in English winter wheat fields

	AG2-1	**Rc**	**OA**	**OY**
***Temp*$_{max}$ (°C)[1]**				
Autumn/winter[a]	ns	0.24 (0.0194)	-0.34 (<0.001)	ns
Spring[b]	ns	0.31 (0.0016)	0.22 (0.0278)	0.45 (<0.001)
Summer[c]	ns	-0.28 (0.0044)	ns	ns
***Temp*$_{min}$ (°C)[2]**				
Autumn/winter	ns	0.28 (0.0052)	-0.37 (<0.001)	ns
Spring	ns	0.36 (<0.001)	ns	0.29 (0.003)
Summer	ns	ns	ns	ns
Total rainfall (mm)				
Autumn/winter	ns	ns	0.30 (0.0026)	ns
Spring	ns	ns	ns	0.20 (0.0469)
Summer	ns	0.30 (0.0025)	ns	ns
Relative humidity (%)				
Autumn/winter	ns	-0.21 (0.035)	ns	ns
Spring	ns	0.21 (0.0331)	0.20 (0.0433)	0.28 (0.0052)
Summer	ns	ns	ns	ns

R. solani (AG2-1) and *R. cerealis* (Rc), *O. acuformis* (OA), *O. yallundae* (OY). ns = not significant (P > 0.05). [1] - *Temp*$_{max}$ - temperature maximum (°C). [2] - *Temp*$_{min}$ - temperature minimum (°C). [a] - Autumn/winter - Pre-sowing to GS 21-31. [b] - Spring - GS21-31 to GS 37-45. [c] - Summer- GS37-45 to GS 65-75.

Table 3.11 Correlation coefficients (*r*) and probabilities (in parentheses) between pathogen DNA concentrations (log pg ng^{-1} of total DNA) in stem bases (5 cm stem basal region) collected at GS21-31, GS37-45 and GS65-75 in 201112 to 2012/13 and temperature (*max* and *min*), total rainfall and relative humidity in English winter wheat fields

	AG2-1	Rc	OA	OY	Mn	Mm
***Temp*$_{max}$ (^{o}C)[1]**						
Autumn/winter[a]	ns	0.49 (<0.001)	0.26 (0.0088)	0.37 (<0.001)	0.45 (<0.001)	0.26 (0.0093)
Spring[b]	ns	0.53 (<0.001)	0.42 (<0.001)	0.38 (<0.001)	ns	ns
Summer[c]	ns	-0.39 (<0.001)	-0.26 (0.0109)	ns	ns	ns
***Temp*$_{min}$ (^{o}C)[2]**						
Autumn/winter	ns	0.52 (<0.001)	0.20 (0.0414)	0.29 (0.0034)	0.40 (<0.001)	0.21 (0.0314)
Spring	ns	0.55 (<0.001)	0.44 (<0.001)	0.36 (<0.001)	ns	ns
Summer	ns	ns	0.21 (0.0362)	ns	ns	ns
Total rainfall (mm)						
Autumn/winter	ns	-0.56 (<0.001)	-0.46 (<0.001)	-0.51 (<0.001)	-0.47 (<0.001)	-0.28 (0.0043)
Spring	ns	0.45 (<0.001)	0.28 (0.0045)	0.33 (<0.001)	ns	ns
Summer	ns	0.44 (<0.001)	0.33 (<0.001)	ns	ns	ns
Relative humidity (%)						
Autumn/winter	ns	-0.47 (<0.001)	-0.38 (<0.001)	-0.39 (<0.001)	-0.21 (0.0333)	-0.24 (0.0157)
Spring	ns	0.33 (<0.001)	0.27 (0.0064)	0.28 (0.0042)	ns	ns
Summer	ns	0.31 (0.0019)	0.21 (0.0392)	ns	ns	ns

R. solani (AG2-1) and *R. cerealis* (Rc), *O. acuformis* (OA), *O. yallundae* (OY), *M. nivale* (Mn), *M. majus* (Mm). ns = not significant (P > 0.05). [1] - *Temp*$_{max}$ - temperature maximum (^{o}C). [2] - *Temp*$_{min}$ - temperature minimum (^{o}C). [a] - Autumn/winter - Pre-sowing to GS 21-31. [b] - Spring - GS21-31 to GS 37-45. [c] - Summer- GS37-45 to GS 65-75.

Table 3.12 Correlation coefficients (*r*) and probabilities (in parentheses) between pathogen DNA concentrations (log pg ng^{-1} of total DNA) in stems (15 cm above the 5 cm basal region) collected at GS37-45 and GS65-75 in 201112 to 2012/ and temperature (*max* and *min*), total rainfall and relative humidity in English winter wheat fields

	Rc	**OA**	**OY**
***Temp*$_{max}$ (°C)[1]**			
Spring[b]	0.64 (<0.001)	0.36 (<0.001)	ns
Summer[c]	ns	-0.37 (<0.001)	-0.26 (0.0074)
***Temp*$_{min}$ (°C)[2]**			
Spring	0.68 (<0.001)	0.38 (<0.001)	0.28 (0.0055)
Summer	0.28 (0.005)	ns	ns
Total rainfall (mm)			
Spring	0.66 (<0.001)	ns	ns
Summer	ns	0.52 (<0.001)	0.39 (<0.001)
Relative Humidity (%)			
Spring	0.46 (<0.001)	ns	ns
Summer	ns	0.37 (<0.001)	0.29 (0.0029)

R. cerealis (Rc), *O. acuformis* (OA), *O. yallundae* (OY). ns = not significant (P > 0.05).
[1] - *Temp*$_{max}$ - temperature maximum (°C). [2] - *Temp*$_{min}$ - temperature minimum (°C). [a] - Autumn/winter - Pre-sowing to GS 21-31. [b] - Spring - GS21-31 to GS 37-45. [c] - Summer- GS37-45 to GS 65-75.

3.3.9 Principal component analysis (PCA) for diseases, pathogen DNA and meteorological variables in soil, roots, stem bases and stems

3.3.9.1 GS 21-31

Associations at GS 21-31 between species and diseases with meteorological variables in autumn/winter period are shown as PCA biplots in Figure 3.1 for soil (a), roots (b) and stem bases (c). In the soil biplot (PC1 and PC2 explained 59% of the variation) vectors for all species, except *M. majus*, were clustered along the Y-axis and showed no association with any of the meteorological variables. The root biplot (PC1 and PC2 = 52%) weakly grouped root rot and *R. cerealis* together. However, the relatively short vector of root rot indicates this factor had a minor influence on the variation in this data set. *Oculimacula* spp. showed no association with the other pathogens or root rot. The stem bases biplot (PC1 and PC2 = 50%) clustered the *Oculimacula* spp. and *Microdochium* spp. with eyespot. Brown foot rot showed independence from the principle causal agent the *Microdochium* spp. Sharp eyespot was clustered with rainfall and humidity but showed a negative association with its causal agent *R. cerealis*.

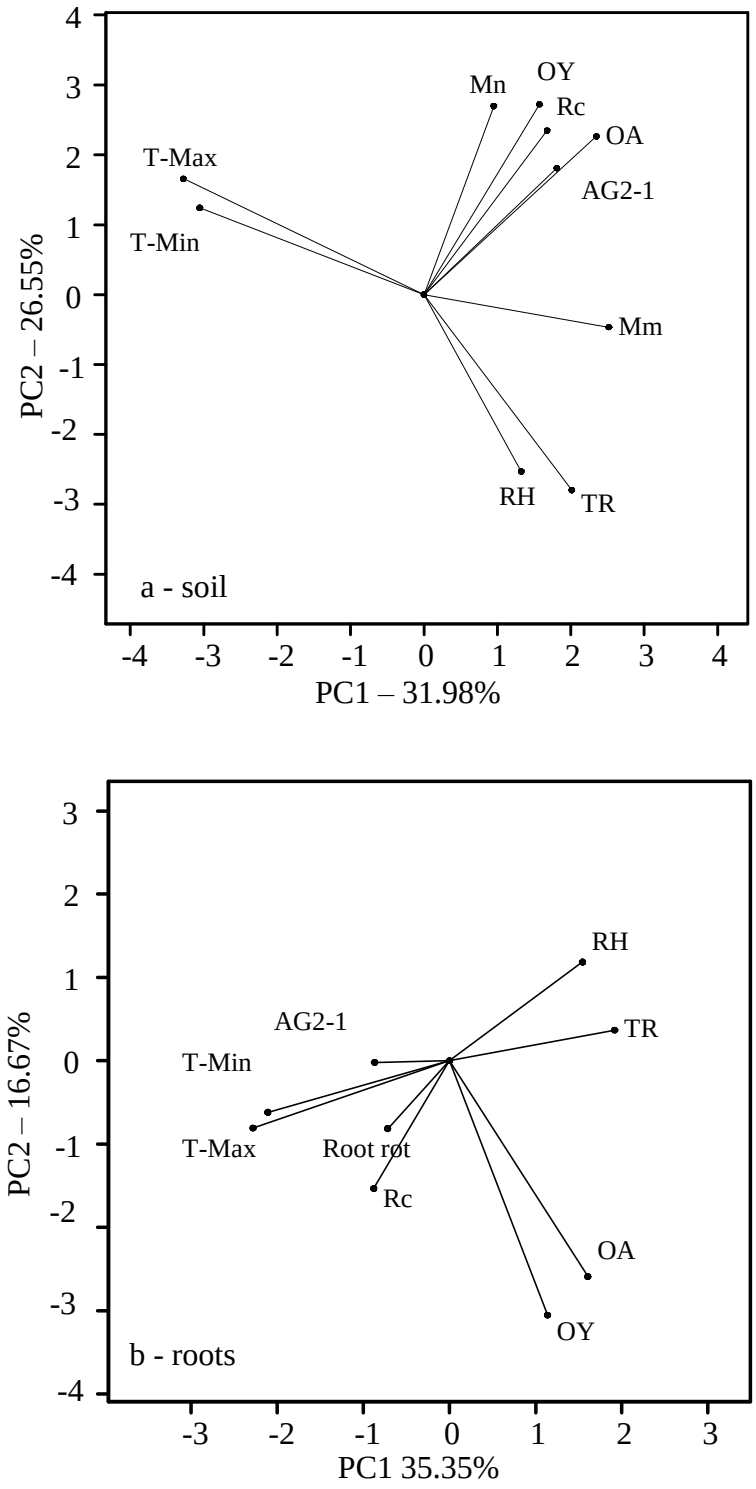


Figure 3.1 Biplot of the principle component analysis showing relationships between pathogens, disease and meteorological variables in a) soil, b) roots and c) stem bases of winter wheat at GS 21-31. *M. nivale* (Mn), *M. majus* (Mm), *O. acuformis* (OA), *O. yallundae* (OY), *R. cerealis* (Rc) , *R. Solani* AG 2-1 (AG 2-1), eyespot, sharp eyespot (S. eyespot) and brown foot rot (BFR) and disease index (DI). Total rainfall (TR), relative humidity (RH) and mean daily maximum (T-max) and minimum temperature (T-min). (*Continued*)

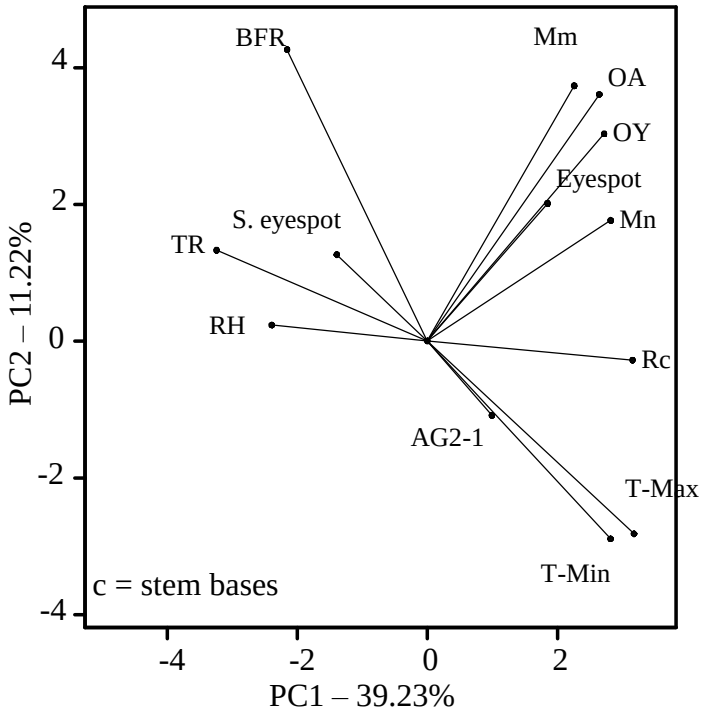


Figure 3.1 Biplot of the principle component analysis showing relationships between pathogens, disease and meteorological variables in a) soil, b) roots and c) stem bases of winter wheat at GS 21-31. *M. nivale* (Mn), *M. majus* (Mm), *O. acuformis* (OA), *O. yallundae* (OY), *R. cerealis* (Rc) , *R. Solani* AG 2-1 (AG 2-1), eyespot, sharp eyespot (S. eyespot) and brown foot rot (BFR) and disease index (DI). Total rainfall (TR), relative humidity (RH) and mean daily maximum (T-max) and minimum temperature (T-min).

3.3.9.2 GS 37-45

At GS 37-45 associations between species and diseases with meteorological variables in the spring are shown as PCA biplots in Figure 3.2 for soil (a), roots (b), stems bases (c) and stems (d). In the soil biplot (PC1 and PC2 = 55%) again all species, except *M. majus,* were clustered together and showed no association with the meteorological variables. In the root biplot (PC1 and PC2 = 54%) none of the species were associated with root rot. However, root rot was clustered with each of the meteorological variables. Associations in the stem bases biplot (PC1 and PC2 = 46%) showed *R. cerealis* and eyespot clustered along with the meteorological variables along the x-axis; the *Oculimacula* spp. to a lesser extent were also aligned with this group. BFR was directionally aligned with the *Microdochium* spp. indicating an association. However, sharp eyespot showed no association with its causal agent *R. cerealis.* In contrast in the stems biplot (PC1 and PC 2 = 70%) *R. cerealis* was clustered with sharp eyespot but also with eyespot and $temp_{max\ \&\ min}$ and rainfall. The *Oculimacula* spp. showed no association with eyespot and also negatively associated with rainfall and humidity. The short BFR vector indicates this factor had a relatively minor influence on the variation in this data set.

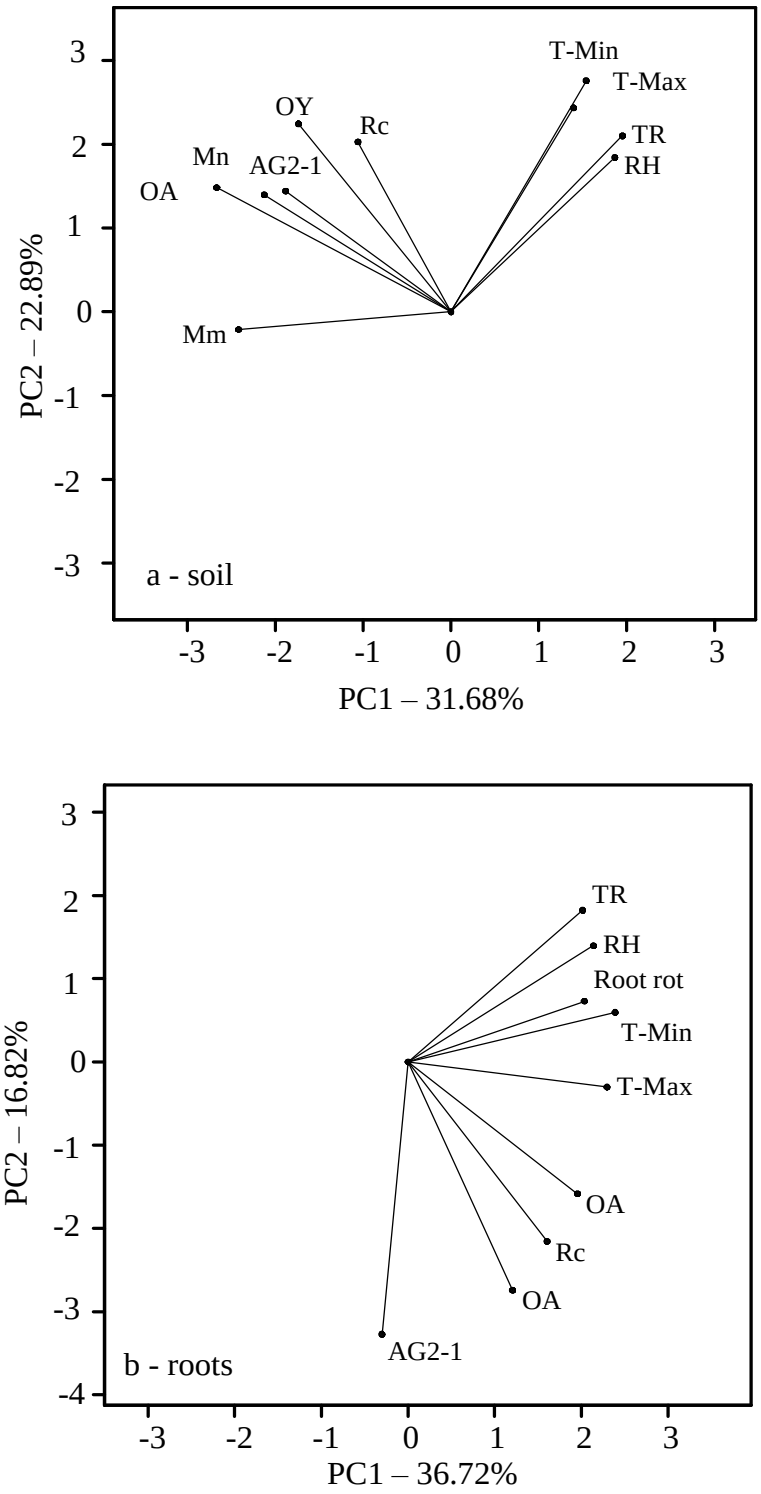


Figure 3.2 Biplot of the principle component analysis showing associations between pathogens, disease and meteorological variables in a) soil, b) roots and c) stem bases and d) stems of winter wheat at GS 37-45. *M. nivale* (Mn), *M. majus* (Mm), *O. acuformis* (OA), *O. yallundae* (OY), *R. cerealis* (Rc) , *R. Solani* AG 2-1 (AG 2-1), eyespot, sharp eyespot (S. eyespot) and brown foot rot (BFR) and disease index (DI). Total rainfall (TR), relative humidity (RH) and mean daily maximum (T-max) and minimum temperature (T-min). (*continued*)

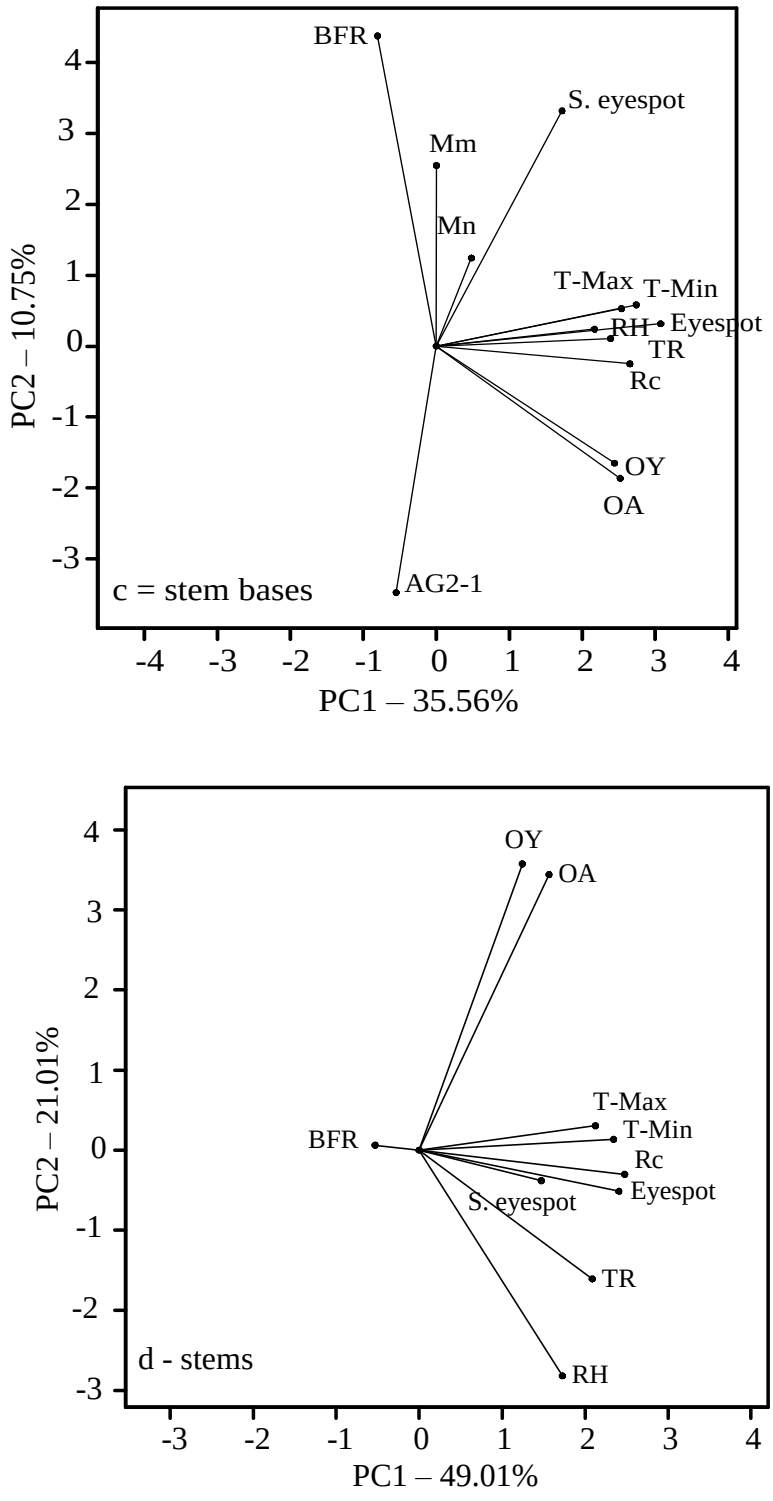


Figure 3.2 Biplot of the principle component analysis showing associations between pathogens, disease and meteorological variables in a) soil, b) roots and c) stem bases and d) stems of winter wheat crops at GS 37-45. *M. nivale* (Mn), *M. majus* (Mm), *O. acuformis* (OA), *O. yallundae* (OY), *R. cerealis* (Rc) , *R. Solani* AG 2-1 (AG 2-1), eyespot, sharp eyespot (S. eyespot) and brown foot rot (BFR) and disease index (DI). Total rainfall (TR), relative humidity (RH) and mean daily maximum (T-max) and minimum temperature (T-min).

3.3.9.3 GS 65-75

At GS 65-75 associations between species and meteorological variables in period 3 are shown as PCA biplot in Figure 3.3 for soil (a), roots (b), stems bases (c) and stems (d). In the soil biplot (PC1 and PC 2 = 56%) all species, except *M. majus*, were again clustered together but also aligned with $temp_{min}$ in period 3. In the roots biplot (PC1 and PC 2 = 53%) root rot was not associated with any of the pathogens. However, again the short vector for root rot suggests this factor had a minor effect on the variation in this data set. The *Oculimacula* spp. and *R. cerealis* were again grouped together suggesting an association between these species in roots. The biplot for stem bases (PC1 and PC2 = 46%) showed eyespot closely aligned to *R. cerealis* than the *Oculimacula* spp. Sharp eyespot was grouped with total rainfall and relative humidity but only loosely associated with its causal agent *R. cerealis*. The *Oculimacula* spp. were aligned with *M. nivale* suggesting an association between these pathogens. In the stems biplot (PC1 and PC2 = 57%) sharp eyespot and eyespot were clustered along the X-axis along with rainfall but only weakly associated with their causal agents. The *Oculimacula* spp. were aligned with *R. cerealis.* DNA of these species showed no association with rainfall or humidity.

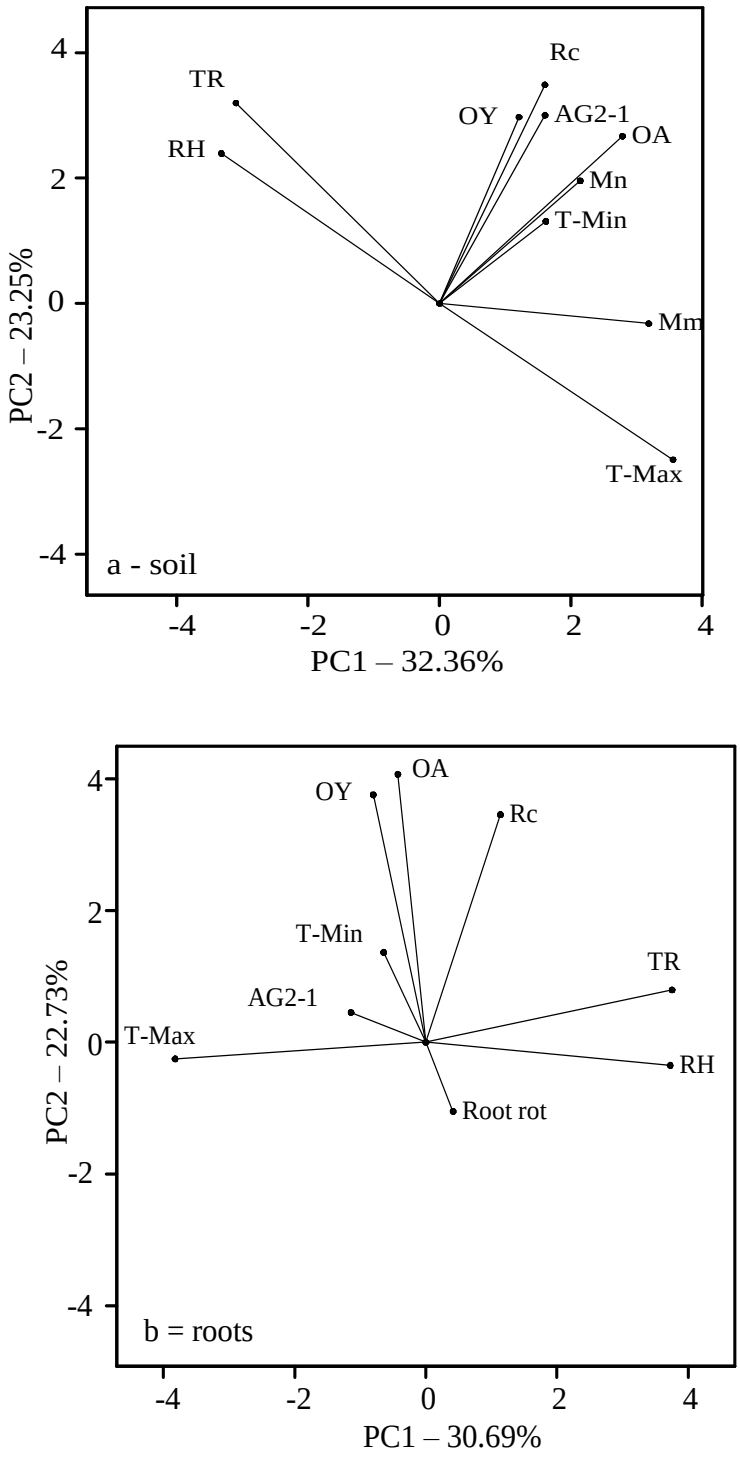


Figure 3.3 Biplot of the principle component analysis showing associations between pathogens, disease and meteorological variables in a) soil, b) roots and c) stem bases and d) stems of winter wheat crops at GS 65-75. *M. nivale* (Mn), *M. majus* (Mm), *O. acuformis* (OA), *O. yallundae* (OY), *R. cerealis* (Rc) , *R. Solani* AG 2-1 (AG 2-1), eyespot, sharp eyespot (S. eyespot) and brown foot rot (BFR) and disease index (DI). Total rainfall (TR), relative humidity (RH) and mean daily maximum (T-max) and minimum temperature (T-min).(*Continued*)

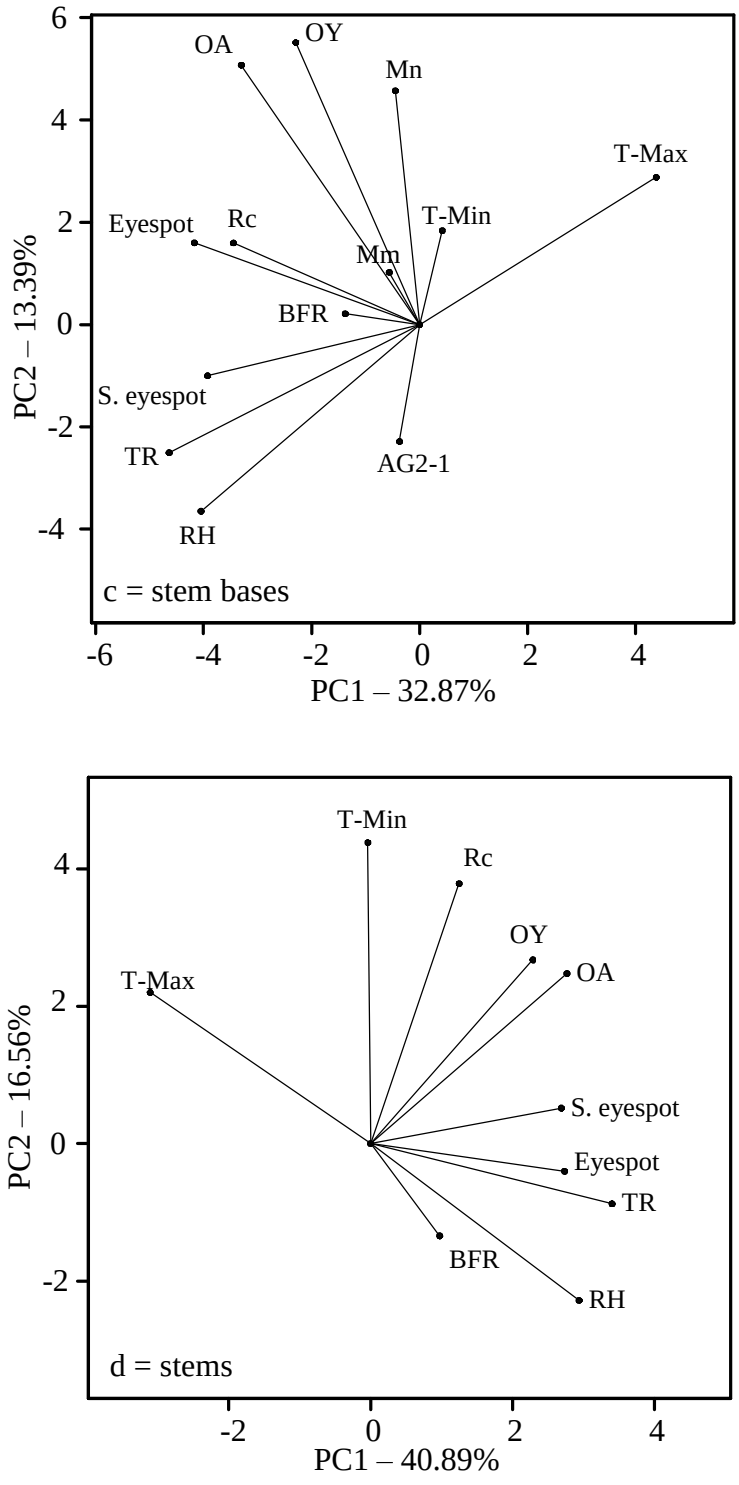


Figure 3.3 Biplot of the principle component analysis showing associations between pathogens, disease and meteorological variables in a) soil, b) roots and c) stem bases and d) stems of winter wheat crops at GS 65-75. *M. nivale* (Mn), *M. majus* (Mm), *O. acuformis* (OA), *O. yallundae* (OY), *R. cerealis* (Rc) , *R. Solani* AG 2-1 (AG 2-1), eyespot, sharp eyespot (S. eyespot) and brown foot rot (BFR) and disease index (DI). Total rainfall (TR), relative humidity (RH) and mean daily maximum (T-max) and minimum temperature (T-min).

3.4 DISCUSSION

3.4.1 Disease occurrence in English wheat crops

Results showed that the majority of wheat crops in England exhibited root rot symptoms of low to moderate severity, with a peak at GS GS21-31, which declined as the plants matured. An earlier sampling in the autumn prior to GS21 may have revealed symptoms of greater severity as young juvenile root tissue is most susceptible to attack by soil borne pathogens (Lockwood, 1986). Symptoms on roots were generally superficial small lesions on the root surface that caused discolouration and limited rotting of the root cortex. However, the classic 'pruning' or 'spear tipping' associated with severe rhizoctonia root rot infection by pathogenic *R. solani* AG's (Weller *et al.,* 1986; Mazzola *et al.,* 1996) were rarely seen in this study. This is probably because the main *R. solani* AG detected was AG 2-1 which is generally only considered weakly pathogenic to wheat (Demirci, 1998; Sturrock *et al.,* 2015). There were also no relationships between root rot and the pathogens in this study in the PCA. This is probably because root rot is generally not caused by a single pathogen but by a complex of species (Harris and Moen, 1985; Raaijmakers *et al.,* 2009). Therefore, a number of other species are likely to be involved in causing the root rot observed in this study.

BFR was the predominant SBD disease throughout the crop growing season in both years of study reaching 100% incidence by GS 37 in English wheat crops. Polley and Thomas, (1991) and Hardwick *et al.* (2001) in studies over longer time periods (≥10 years) also showed BFR was the most commonly occurring stem disease in UK wheat crops, found in ≥74 and ≥89% of crops sampled respectively. Therefore, the incidence in this study was slightly higher than the previous studies mentioned. The only correlations with meteorological variables were those over the autumn/winter period suggesting BFR was more severe in cooler and wetter areas. This was shown at GS 21-31 when BFR index was 68% higher in 2012/13 when conditions were wetter and cooler than 2011/12 (Table 3.7). BFR remained relatively constant at GS 37-45 and GS 65-75 between seasons, suggesting variation in environmental conditions had little effect on the development of disease as the crop matured. This was shown by the lack of any associations between BFR and with meteorological variables at GS 37-45 and GS

65-75. It is generally considered that warmer and drier summer conditions are conducive to BFR (Bateman, 1993; Bateman *et al.,* 1998). However, in this study there was no difference in BFR when there was contrasting amounts of rainfall in the summer periods.

In contrast eyespot and sharp eyespot showed considerable season to season variation. The incidence and severity of eyespot and sharp eyespot were higher in 2011/12 compared to 2012/13; this was associated primarily with differences in environmental conditions. Eyespot and sharp eyespot at GS 37-45 and GS 65-75 were correlated with higher temperatures and rainfall in the spring but lower temperatures and again higher rainfall in the summer. In 2011/12 there were larger amounts of rainfall and higher temperatures in the spring and more rainfall and lower temperatures in the summer compared to 2012/13 (Table 3.7). Studies have shown that rainfall and temperature are the two most important environmental factors influencing eyespot infections (Fitt *et al.,* 1988; Bock *et al.,* 2009; Matusinsky *et al.,* 2009). Rainfall is important for lesion establishment on mature stems (McCartney and Fitt, 1998) and crop desiccation since dry periods allow outer leaf sheaths to shed resulting in inoculum loss to cause further eyespot infections on the stem. In addition, eyespot incidence and severity have also been shown to increase linearly with thermal time (Wan *et al.,* 2005; Bock *et al.,* 2009). Therefore, the environmental conditions in 2011/12 were more conducive to eyespot development than 2012/13. Previous research on sharp eyespot has shown the disease is favoured by dryer soil conditions with incidence on wheat seedlings decreasing with increasing soil moisture content (Herman *et al.,* 1992b; Kalberer and Gisi, 1997). Cooler temperatures are also known to influence sharp eyespot development with the optimal temperature for infections around 9-10°C (Wiese, 1987; Parry, 1990). Burpee *et al.* (1980b) found sharp eyespot on wheat plants decreased as temperatures increased from 16 to 28°C. These reports suggest sharp eyespot is favoured by cooler and dryer conditions. However, in this study total rainfall in the spring and summer of 2011/12 were higher than in 2012/13. This suggests rainfall was important for the development of sharp eyespot in this study. In the following season sharp eyespot failed to develop probably due to excessive rainfall in the autumn/winter period which may have prevented early infections. Pitt (1964) stated that early

infections are required for severe infections to manifest at later stages. Eyespot also failed to develop in 2012/13 but between GS 37-45 and GS 65-75 when total rainfall was only 34 mm compared to 180 mm in 2011/12. Rainfall is considered the most important factor in the dispersal of conidia and subsequent disease development (Fitt and Bainbridge, 1983). This would explain the lack of eyespot development in 2012/13.

3.4.2 Population dynamics of pathogens in field soil

This is the first study to investigate the population dynamics of a range of species in the soil of English wheat crops and as such provides new information on the incidence of economically important wheat pathogens in soil. The results showed that *O. acuformis* was the most frequently detected species occurring on average throughout both seasons in 73% of soil samples, followed by *O. yallundae* (68%), *R. solani* AG 2-1 (63%) and *R. cerealis* (54%). Therefore the most widely distributed SBD pathogens in the soil of wheat crops were *Oculimacula* spp. and *R. cerealis* suggesting that the majority of crops will be at risk from their associated diseases eyespot and sharp eyespot, respectively. The predominant *Rhizoctonia* spp. in soil of English wheat crops was *R. solani* AG 2-1 occurring on average in 63% of fields. This finding is in contrast to a previous *Rhizoctonia* soil survey. Goll *et al.* (2014) collected soil samples (n = 60) from arable fields across the UK and found AG 2-1 in just 13% of samples using a soil baiting method. However, this study identified species by extracting DNA from soil followed by species specific real-time PCR assays which is far more sensitive method than soil baiting. This could explain the higher percentage of fields containing AG 2-1 in this study. Previous studies in wheat growing regions of America (Schroeder *et al*., (2011) and in potato crops in south eastern Australia (Sparrow *et al.*, 2015) have also shown AG 2-1 to be the predominant AG of *R. solani* in soil samples. This demonstrates the widespread distribution of this species not just in the UK but also globally. However, reports of AG 2-1 as a pathogen of wheat are contrasting with some showing it is pathogenic (Roberts and Sivasithamparam, 1986) and other suggesting it is only weakly or not pathogenic (Sweetingham *et al.*, 1986; Khangura *et al.*, 1999). Currently less is known of the aggressiveness of UK AG 2-1 isolates to wheat in England but

since the pathogen has a broad host range and it is highly pathogenic to OSR (Verma, 1996; Babiker *et al.,* 2013) the implications such as yield losses from AG 2-1 to OSR in the arable rotations could be significant.

In this study *R. cerealis* was also widespread occurring in 54% of soil samples. Goll *et al.* (2014) failed to detect any *R. cerealis* not just in UK soil samples but in samples collected from 136 locations across Europe. This suggests soil baiting used by Goll *et al.* (2014) is not suitable to detect *R. cerealis.* Goll *et al.* (2014) found AG 9 was the predominant *Rhizoctonia* spp. in UK arable fields, identified in 34% of fields. In addition, they also detected AG 5 (9%) and AG 8 (3%). These species are all known pathogens of potatoes (Yanar *et al.* 2005; Woodhall *et al.,* 2007). In this study potatoes did not feature in the rotation of the sampled fields. This could explain the absence of AG 9 and the lower incidence of AG 5 (2%) and AG 8 (2%) in this study. *R. solani* AG 5 has been shown to vary in pathogenicity to wheat (Ogoshi *et al.*, 1990; Rush *et al.,* 1994; Woodhall *et al.,* 2007). *R. solani* AG 8 is generally considered the main AG causing disease in wheat globally and is a major problem in Pacific Northwest of America and in Australia (MacNish and Neate, 1996; Paulitz *et al.*, 2002). In the UK, AG 8 is known to cause barley stunt disease disorder (Burton *et al.,* 1988; Hide and Firmager, 1990) but this disease occurs infrequently. The results of this study concur that AG 5 and AG 8 occur infrequently and are unlikely to be a major problem in wheat production except in specific areas. There was also no AG 4 HG-I, -II, -III detected in soil samples. This is in agreement with Goll *et al.* (2014) who also failed to identify these species in UK soils.

The use of species specific real-time PCR assays allowed the accurate quantification of targeted pathogens (Mumford *et al.,* 2006) to investigate pathogen population dynamics. In terms of the amount of DNA found in soil samples *R. solani* AG 2-1 was the predominant species throughout the period of study. DNA levels of *O. yallundae* and *R. cerealis* were also relatively high compared to those of the *Microdochium* spp. and *O. acuformis.* Therefore, while *O. acuformis* was the most commonly occurring species in soil samples its DNA concentration were lower than the two *Rhizoctonia* spp. and of *O. yallundae* at each time point sampled. This shows the advantage of real-time PCR to identify pathogen populations in soil samples and accurately quantify species. However,

the PCA showed all pathogens except *M. majus* were positively grouped together at each sampling period. This shows these species were positively associated in the soil but also suggests that no or limited interspecific competition occurred between these species. Changes in the biomass of species showed AG 2-1 in soil generally declined over the season. This suggests a lack of suitable substrate in the soil to sustain the continued accumulation of this pathogen over the growing season. In field soil pathogenic *R. solani* attack their hosts releasing enzymes that break down tissues, thus host infection gives rise to more inoculum (Okubara *et al.,* 2014). This would suggest winter wheat is not a major host for AG 2-1 in English wheat fields or it has a short susceptibility period most likely at seedling stage.

Overall *M. nivale* and *M. majus* were the least detected species in terms of incidence and biomass. The incidence of *M. nivale* and *M. majus* increased in both seasons. This was most notable for *M. nivale* over the autumn/winter period when incidence increased by >100% in both seasons. It is generally thought that seed borne inoculum is the most important source of inoculum (Parry *et al*.,1995). The increasing incidence after pre-sowing suggests these pathogens may have been imported into fields on infected seed. Biomass of both species fluctuated in soil samples between sampling periods but was generally higher at GS 65-75 than at pre-sowing. Previous research in the Netherlands monitoring DNA levels of *M. nivale* on infected wheat residues on the soil surface under field conditions showed the species peaked in September then declined until the following June (Kohl *et al.,* 2007). This is the only previous study to show changes of *M. nivale* over a similar time period in semi-comparable conditions to this study. However, comparisons are limited due to the different extraction methods (plant *vs.* soil) and limited number of sites (two) in Kohl *et al.* (2007) study.

The associations between meteorological variables and pathogen DNA in soil were weak or showed no consistent trend across sampling periods. This suggests changes in DNA are more likely to result from differences in the way these species interact with the host, other soil microflora or availability of substrate in the soil than due to interactions with the environment. Okubara *et al.* (2014) showed that DNA concentrations of AG 8 in field soil were negatively correlated

with total rainfall in Washington State, America. This suggests there are differences in how the various *Rhizoctonia* spp. in soil respond to meteorological variables.

3.4.3 Population dynamics of pathogens in root tissue

The population dynamics in roots showed that the most commonly occurring species were *O. yallundae* (90%) and *O. acuformis* (88%). These were followed by *R. cerealis* (56%) and *R. solani* AG 2-1 (52%). This is the first study to show incidence of these species on wheat roots in England. However, while AG 2-1 was clearly the predominant species in soil, it was only detected on roots in trace amounts suggesting wheat is not likely a major host. Indeed, there are contrasting reports of AG 2-1 causing disease on wheat roots. Roberts and Sivasithamparam, (1986) isolated AG 2-1 from damaged wheat roots from within bare patches in Australia and found the pathogen to cause considerable disease on wheat roots. In contrast Tewoldmedhin *et al*. (2006) in South Africa isolated AG 2-1 from a range of crops and found isolates to be non-pathogenic on wheat. This suggests there is large variation in the pathogenicity of isolates of AG 2-1 which could be due to how isolates have evolved under contrasting environments/hosts. In addition, although *R. cerealis* DNA was frequently detected (56%) on roots previous research has shown that this species did not cause any disease on wheat roots (Lipps and Hear. 1982; Sweetingham *et al*., 1986). It is not known what role, if any, the occurrence of *R. cerealis* or *Oculimacula* spp. on roots has on the disease epidemiology of sharp and true eyespot or what affect root infection by these pathogens would have on the plant and ultimately yield. However, DNA of each species at GS 21-31 was only detected in trace amounts and differences in DNA concentrations between pathogens only became apparent in the last two sampled growth stages. Lockwood (1986) stated that juvenile root tissue is most susceptible to attack. However, in this study root rot declined with crop development while pathogen DNA detected on roots increased, suggesting these species were not major causal organisms of wheat root rot disease. In addition, pathogen DNA in roots generally showed no or weak associations with meteorological variables which were shown in the correlations and PCA. However, the *Oculimacula* spp. and *R. cerealis* were grouped together in the root

PCA at GS 37-45 and GS 65-75 suggesting a positive association between these species on roots. However, caution is required when interpreting these results. The destructive nature of removing plants from field soil results in only a small proportion of the root system been recovered and weakened diseased roots, that easily break, often remain in the soil profile. Therefore, the impact of soil borne pathogens on roots is likely to be underestimated.

3.4.4 Population dynamics of pathogens in stem tissue

The most frequently occurring species on stem bases were *Oculimacula* spp. and *Microdochium* spp. in ≥90% of crops at each growth stage in both seasons. Previous studies identified *O. acuformis* as the predominant *Oculimacula* spp. in UK wheat crops (Polley and Turner, 1995; Turner *et al*, 1999; Nicholson and Turner, 2000; Turner *et al.* 2001). However, in this study *O. acuformis* and *O. yallundae* occurred together in wheat crops at each growth stage sampled in both seasons. The stem base and stem PCA also clearly grouped the *Oculimacula* spp. together at each growth stage indicating a strong association. This suggests the population structure of *Oculimacula* spp. has changed and that both species now co-exist in the majority of crops. The populations of these species have altered previously; in the 1980's *O. yallundae* dominated whilst in the 1990's *O. acuformis* was the predominant species, this was thought to be due to differences in fungicide sensitivity (King and Griffin, 1985; Polley and Turner, 1995; Nicholson and Turner, 2002). However, past studies have generally used isolations (Polley and Turner, 1995) or conventional PCR (Turner *et al*, 1999; Nicholson and Turner, 2000; Turner *et al.* 2001) to detect the predominant species. The use of species specific real-time PCR assays may, for the first time, have allowed more accurate picture of the population dynamics of the *Oculimacula* spp. Real-time PCR assays are known to be 100 times more sensitive than conventional PCR (Ratti *et al.* 2004). However, *O. yallundae* clearly had the highest DNA concentrations of the two eyespot species throughout the period of study indicating that any shift in pathogen population was in favour of *O. yallundae*. This was most notable at GS65-75 in 2012/13 when DNA concentrations of *O. yallundae* were 24.2-fold higher than *O. acuformis*. It is known that *O. yallundae* has a higher reproductive rate and is able to penetrate leaf sheaths quicker than *O. acuformis* thus reaching the stem earlier

in the season before outer leaf sheaths are lost to senescence (Bierman *et al.* 2002; Wan *et al.* 2005). This may explain the higher amounts of *O. yallundae* DNA in samples.

The *Microdochium* spp. were also widespread occurring in 90-100% of stem bases which is considerably higher than the occurrence in soil. Humphreys *et al.* (1995) found that seed infected with *Microdochium* spp. was effectively transmitted to seedlings. This adds further evidence that seed borne inoculum is an important source of inoculum in English wheat crops as suggested by Parry *et al*. (1995). In terms of DNA concentrations *M. nivale* was the predominant species in stems bases at GS 21-31, especially in 2011/12. Therefore, this species was able to colonise the stem base over the autumn/winter period to a greater extent than the other pathogens. Mahuku *et al*. (1998) stated that *M. nivale* can be active at temperatures slightly above freezing. This will have provided a competitive advantage over other species, explaining the higher levels found at GS 21-31 (2011/12). However, in the following season all pathogen DNA concentrations in stem bases were low. Negative correlations were also shown between pathogen DNA in stem bases and total rainfall over the autumn/winter period for all species. *Microdochium* spp. are known to cause greater seedling blight in cold but dry soils (Millar and Colhoun 1969). In addition, the survival of *R. cerealis* was found to be inhibited in wet soils (70% maximal water holding capacity) compared to dry soils (30-50% MHC) in a controlled environment study (Herman 1992b). Rainfall over the autumn/winter of 2012/13 was 121% higher than in 2011/12 which may have restricted the development of the species in this study resulting in the low DNA concentrations at GS 21-31.

The simultaneous occurrence of *Oculimacula* spp., *Microdochium* spp. and *R. cerealis* in samples suggests that each species occupies a slightly different niche which enables them to co-exist within the complex (Fitt *et al.* 2006). Bateman (1993) reported that *Oculimacula* spp. were co-isolated with *M. nivale* more frequently than would be expected by chance. In this study the stem base PCA at GS 21-31 and GS 65-75 grouped *Oculimacula* spp. and *Microdochium* spp. together indicating a strong association between the species. However, the PCA at GS 37-45 showed no clear association between these species. Therefore, this positive relationship was not consistent throughout the season. However, in this

study the 36 plant samples were pooled together for DNA extractions and therefore the sequence of infections on individual stems within crops is unknown.

Sharp eyespot symptoms are known to occur up to the fourth internode above the soil (Goulds and Polley, 1990). This study extracted DNA from sections of the stem that were 15 cm above the lower 5 cm basal region (stem base). In both seasons the amount of *R. cerealis* DNA at GS 65-75 was higher up in the stems than stem bases. This confirms that the stem region is a more favourable environment or preferred niche for *R. cerealis.* Good positive associations between *R. cerealis* DNA in stems and $Temp_{max\&min}$ and total rainfall were shown at GS 37-45 although no such correlations were found at GS 65-75. This could suggest a combination of a different micro-climate and a more preferable niche higher up the stem favours the development of *R. cerealis* in this part of the plant. The *Oculimacula* spp. DNA was also quantified in stems but generally at lower levels than *R. cerealis.*

In this study visual disease assessments of stems generally did not agree with the detection of pathogen DNA at the early growth stages. Mixed infections SBD's are known to be difficult to distinguish at early growth stages (Polley and Turner, 1995; Turner *et al.* 2001). Real-time PCR generally detected pathogen DNA at a higher frequency than visual observations identified disease symptoms, especially at GS 21-31 when plants can be asymptomatic. Previous studies have also shown that disease assessments and pathogen diagnoses using PCR are often not in agreement (Turner *et al.* 1999; Turner *et al.* 2001). This shows the advantage of using molecular methods to detect the presence of pathogens at early growth stages when disease assessments can be unreliable. It was also shown that the frequency at which SBD pathogens were detected in soil samples was generally lower than the occurrence in stem bases. This contrasts with *R. solani* AG 2-1 that was detected more frequently in soil samples than plant samples. In the future, growers and agronomists are likely have access to field based molecular tools such as loop-mediated isothermal amplification (LAMP) assays to identify pathogens (Tomlinson *et al.,* 2010). Therefore, the substrate which DNA is extracted from will be important for accurate identification of pathogens.

3.5 CONCLUSIONS

The results within this chapter enable the following conclusions to made:

i. Root rot symptoms were only found at low to moderate severity and showed a declining trend as plants matured.

ii. BFR was the most commonly occurring SBD in English wheat crops in both seasons.

iii. Eyespot and sharp eyespot incidence and severity was higher in 2011/12 than 2012/13 due to differences in environmental conditions.

iv. The most commonly occurring species in soil samples was *O. acuformis* (73%) and *O. yallundae* (68%).

v. The most common occurring anastomosis group of *R. solani* was AG 2-1 found on average in 63% of soil samples.

vi. *R. solani* AG 2-1 was the dominant species in terms of DNA concentrations in soil samples throughout the period of study.

vii. *M. nivale* and *M. majus* were the least occurring species in soil samples in terms of incidence and biomass but their frequency showed an increasing trend in both seasons.

viii. The most commonly occurring species on roots were *O. yallundae* (90%) and *O. acuformis* (88%).

ix. There was no association between pathogen DNA in roots and root rot symptoms, suggesting other causal organisms of symptoms on wheat.

x. The *Oculimacula* spp. and *Microdochium* spp. were frequently detected on stem bases occurring in >90% of samples.

xi. The population structure of the *Oculimacula* spp. has changed with both species occurring together at each growth stage sampled. However, in terms of biomass *O. yallundae* predominates.

xii. The *Microdochium* spp. was found at a considerably higher frequency in stem bases than soil samples.

xiii. The preferred niche for *R. cerealis* was higher up the stem above the stem base but still co-exist along with *Oculimacula* spp. and *Microdochium* spp. on the stems of wheat.

Chapter 4

Effect of agronomy practices and meteorological factors on *Rhizoctonia* spp., *Oculimacula* spp. and *Microdochium* spp. in English wheat crops

4.1 INTRODUCTION

This chapter describes the effect of common agronomy practices and meteorological variables on the incidence and severity of root rot and stem base diseases (SBD) and on the DNA of their associated pathogens. In addition, the effect of agronomy and meteorological variables on *R. solani* AG 2-1 is also described. Agronomy information (e.g. sowing date) of the wheat fields described in the previous chapter was collected from growers/agronomists. Residual maximum likelihood (REML) analysis was used to model agronomy along with meteorological factors on disease and pathogen DNA.

Agronomy decisions can have positive or negative impact on crop disease development. For example, growing of a non-host (e.g. OSR) prior to a host (e.g. wheat) can decrease the incidence of eyespot and sharp eyespot by reducing the amount of primary inoculum available to cause infections (Slopek and Labun, 1990; Colbach *et al.*, 1997b). This shows the value of rotations to manage diseases. Cultivation can also have contrasting effects on the SBD complex. The incidence and severity of BFR was reduced in ploughed experimental plots compared to tined/disc cultivation (Bateman *et al.*, 1998). This was thought to be due to inoculum being buried thus reducing infection potential. In contrast, experimental plots that were ploughed had a higher incidence of eyespot and sharp eyespot compared to tined cultivation (Prew *et al.*, 1995). The authors suggested infected debris returned to the surface is a more potent source of inoculum than infected fresh debris. These studies show how the same agronomy practice can have contrasting effects on the SBD complex. Sowing date also influences the incidence and severity of SBD diseases in winter wheat crops. Hardwick *et al.* (2001) conducted a survey of 360 to 450 fields in England and Wales at GS 73-75 from 1989 to 1990. Sowing the crop in early September resulted in the highest number of tillers with moderate/severe eyespot lesions and November the lowest. However, there is a risk that soil conditions become unconducive to field operations if sowing is delayed into late October and November. Therefore, it is unlikely that growers would delay sowing specifically to control SBD's.

Chemical treatments are commonly used to protect seeds and seedlings against soil-borne or seed-borne pathogens such as the *Microdochium* spp. Research has shown that seed treatments reduce seedling blight by limiting pre and post-emergence damping-off and therefore increase establishment (Haigh *et al.,* 2009). Fungicides are also used to control the pathogens that cause eyespot and sharp eyespot. However, eyespot is the only SBD that is regularly controlled with broad spectrum fungicides with applications targeting eyespot generally applied at GS 30-32 (Burnett *et al.,* 2012). Fungicides commonly used to control eyespot include prothioconazole and boscalid (Burnett *et al.,* 2012). Cyprodinil has also been shown to be effective in reducing eyespot index in winter wheat (Ray *et al.,* 2004). In contrast, fungicides are rarely used to control sharp eyespot as they are not considered to be cost effective. This is probably because chemical control of sharp eyespot is often variable (Parry, 1990; Ray *et al.,* 2004).

Environmental factors also influence the development of fungal pathogens in soil and *in planta* and can consequently alter root and stem base disease severity. Moisture is required for eyespot infections to occur (Fitt *et al.,* 1988) as sporulation of ascospores and conidia is dependent on moisture (Fitt and Bainbridge, 1983). Rainfall is also an important factor that enables splash dispersal of conidia to infect wheat stems (Fitt and Bainbridge, 1983). *Microdochium* spp. are favoured by low temperatures and dry soils and can cause disease even in freezing conditions (Millar and Colhoun, 1969; Haigh and Hare, 2012). However, the two species vary in their ability to cause seedling blight at different temperatures. Seeds artificially inoculated with a conidial suspension showed that *M. nivale* caused consistent seedling blight at 5°C and 15°C compared to *M. majus* that failed to cause disease at 5°C and was only weakly pathogenic at 15°C (Imathiu *et al.,* 2010).

Rhizoctonia solani AG 2-1 was identified as the predominant anastomosis group of *R. solani* in the English wheat fields in the previous chapter. However, in England it is not known what effect agronomic practices are having on AG 2-1 as there have been no previous studies investigating this AG. *Rhizoctonia solani* AG 2-1 is generally considered a *Brassica* pathogen and is known to cause losses in canola production in Canada (Verma, 1996; Babiker *et al.,* 2013). Field experiments in Canada have shown early sowing of canola seed resulted in

greater emergence and higher seed yield relative to late-sown seeds (Hwang *et al.,* 2014). Environmental factors are also known to influence AG 2-1. Greater activity of AG 2-1 at lower temperatures has been shown in growth chamber studies. Yitbarek *et al.* (1988) showed AG 2-1 was highly virulent to canola causing considerable damping-off at soil temperatures <10°C compared to AG 4. There has also been researching showing that minimum tillage rather than ploughing favours the anastomosis groups of *R. solani.* Minimum tillage disrupts the hyphal networks less compared to ploughing which buries the inoculum (MacNish, 1985; Pumphrey *et al.* 1987; Smiley *et al.* 1996). In addition, minimum tillage also encourages volunteer plants and grass weeds that *R. solani* can survive on in between host crops (MacNish, 1985; Pumphrey *et al.* 1987; Smiley *et al.* 1996).

The identification of agronomic practices that influence the occurrence and the development of diseases can enable a set of risk assessments to be produced. For example, there is a risk assessment for eyespot produced by the Agricultural and Horticultural Development Board (AHDB). Growers can assess the risk of eyespot based on a points system depending on area, soil type, previous crop, tillage and sowing date (Burnett *et al.*, 2012). This enables growers to calculate the risk to the growing crop and therefore if specific treatments are justified.

The aim of this study was to determine the effects of common agronomy practices and meteorological variables that occurred over the autumn/winter period on root rot and SBDs and their associated pathogens and *R. solani* AG 2-1 in winter wheat crops.

The individual objectives were to:

i) Identify agronomic practices and meteorological variables that influence the severity of root rot and the SBD complex.

ii) Investigate agronomic practices and meteorological variables that influence pathogen biomass (DNA) in soil and *in planta*.

The specific null hypotheses tested within this chapter are:

i) There will be no effect of agronomic practices or meteorological variables on root rot and the SBD complex.

ii) There will be no effect of agronomic practices or meteorological variables on pathogen biomass (DNA) in soil and *in planta.*

4.2 MATERIALS AND METHODS

4.2.1 Soil and plant sampling

Refer to section 3.2.1 for full method details.

4.2.2 Visual disease assessments

Refer to section 2.4 for full method details.

4.2.3 Soil and plant DNA extractions

Refer to section 2.2.1 (soil) and 2.2.2 (plant) for full method details.

4.2.4 Agronomy factors

A questionnaire was completed by growers/agronomists to gather information on a set of predetermined common agronomy practices that were performed on each individual field sampled in 2011/12 and 2012/13. Tables 4.1 and 4.2 presents the agronomy practices (factors) growers/agronomists were asked to provide information about and the grouping of responses (factor level).

4.2.5 Meteorological data

Section 3.2.5 provides full details about how the meteorological data was gathered. In this chapter meteorological data was only used from the date crops were sown until the first sampling period for plants (GS 21-31). Therefore data was gathered only for the autumn/winter period of this study. The meteorological variables investigated were mean daily maximum ($Temp_{max}$), minimum ($Temp_{min}$) temperature (°C), total rainfall (mm) and mean daily relative humidity (%). Each sampled location was assigned to a $Temp_{max\&min,}$ total rainfall and relative humidity group based on the meteorological data over the autumn/winter period (Table 4.3). Therefore, this chapter describes the effect of meteorological

variables over the autumn/winter on the development of root rot, SBD's and pathogen DNA in soil and *planta* at GS 21-31, GS 37-45 and GS 65-75.

4.2.6 Statistical analysis

All statistical calculations were done using Genstat (17^{th} edition) statistical package (Lawes Agricultural Trust, Rothamsted, UK). All DNA data was $\log_{10}$ transformed. To estimate the effects of agronomic factors on root rot, SBD's and pathogen biomass (DNA) over the growing season the data was subjected to repeated measurements in residual maximum likelihood (REML) (also called restricted maximum likelihood). REML allows repeated measurements over time to be analysed for unbalanced data structures like in this study where, for example, there is an uneven distribution of fields in each season. Fields were categorized into groups based on the agronomy information gathered from growers and agronomists (Tables 4.1 and 4.2) in order to examine the effects of agronomic practices. Therefore, fields were grouped based on the explanatory variables which are shown in Appendix 4 along with their REML codes. REML models were produced for the effect of agronomic and meteorological variables on the development of root rot, eyespot, BFR and sharp eyespot at GS 21-31, 37-45 and 65-75. Models were also produced for the effect of agronomic and meteorological variables on the development of pathogen DNA in soil, roots and stem bases at GS 21-31, 37-45 and 65-75. In the models growth stage is presented as time (T). REML models were designed by initially fitting all explanatory variables. These were then eliminated one at a time if they were non-significant ($P \leq 0.05$) until only significant variables remained. Explanatory variables only remained in the model if significant when fitted as first and last terms.

Table 4.1 Agronomical information gathered for English wheat fields sampled in 2011/12 and 2012/13. In parenthesis [-] number of observations for each factor

Factor	Factor level	Description/comments	Reference
Soil texture	Silty clay loam [11], sandy clay loam [52], clay/clay loam [24], sandy loam [15]	Soil texture was determined by a combination of grower's interpretation, ribbon test and Landis soil maps.	Landis, not dated; Brady and Weil (2008)
Previous crop	Winter wheat [33], continuous wheat [10], OSR [43], Legumes (peas or beans) [6], Other cereal (oats or maize) [10]	Previous crop is defined as the crop grown in the field in the season prior to sample collection.	
Position of wheat in the rotation	1st wheat [59], 2nd wheat [33], or continuous wheat (CW) [10]	Crops were classified 1st wheats when previous crop grown in the field was not wheat. 2nd wheats when prior crop grown was wheat. Continuous wheat were wheat was grown for >5 years in the field	
Cultivation	Minimum tillage [57], ploughing [45]	Minimum tillage is defined as disc based non-inversion tillage. Ploughing (mouldboard) is were soil was inverted to depths of 20-25 cm	
Eyespot resistance ratings (ER)	ER - 4 [26], 5 [45], 6 [20], and 8 [11]	The varieties of the wheat crops sampled were grouped based on their ER rating on a scale of 1-9, high figure indicates variety had a high degree of resistance.	AHDB, not dated (c&d)
Sowing date	1st-14th Sept [18], 15th-30th Sept [22], 1st-14th Oct [40], 15th-31st Oct [15], 1st > Nov [7]	The time period in which crops were sown.	

Table 4.2 Active ingredients applied as seed treatments or T1 (GS31-32) and T2 (GS39) foliar fungicides to English wheat crops sampled in 2011/12 and 2012/13. In parenthesis [-] number of observations for each factor

Factor	Factor level	Description/comments	Reference
Seed treatment	Prothioconazole (100 g a.i l^{-1}) [27] Prothioconazole (50 g a.i l^{-1}) + clothianidin (250 g a.i l^{-1}) [43] Prothioconazole + clothianidin + silthiofam (125 g a.i l^{-1}) [12] Silthiofam (125 g a.i l^{-1}), [5] Fludioxonil (25 g a.i l^{-1}) & silthiofam (125 g a.i l^{-1}) [9] Other [6] (Prothioconazole (50 g a.i l^{-1}) + clothianidin (250 g a.i l^{-1}) fluquinconazole (167 g a.i l^{-1}) fludioxonil (25 g a.i l^{-1}) + difenoconazole (25 g a.i l^{-1})	Seed treatments were grouped to investigate differences between active ingredients. For example, is there extra activity from prothioconazole + clothianidin + silthiofam in mixture compared to silthiofam alone. Active ingredients with insufficient degrees of freedom for meaningful statistical analysis were grouped together (group name - other).	
T1 fungicide (GS 31-32)	MSI + triazole [9] MSI + prothioconazole + strobilurin [9] SDHI + triazole + other [8] Prothioconazole + other [29] Boscalid + other [44]	T1 fungicides were grouped to separate active ingredients with activity to eyespot (e.g. prothioconazole and boscalid) to compare differences in control. Rates applied were standard recommended agronomy rates.	Lainsbury (2014)
T2 fungicide (GS 39)	Epoxiconazole + strobilurin [6] Epoxiconazole + strobilurin + SDHI [7] Epoxiconazole + triazole + other [36] MSI + triazole + other [8] Prothioconazole + other [35] Strobilurin + other [7]	T2 fungicides were grouped to investigate any difference in control of eyespot, BFR and sharp eyespot between the active ingredients. Rates applied were standard recommended agronomy rates.	Lainsbury (2014)

Table 4.3 Temperature (*minima* and *maxima*) (°C), total rainfall (mm) and relative humidity (%) for the locations of English wheat crops sampled in 2011/12 and 2012/13. In parenthesis [-] number of observations for each factor.

Factor	Factor level	Description/comments	Reference
Mean $Temp_{min}$ (^{o}C)	<2.99 [9], 3-3.99 [41], 4-4.99 [37] or 5> [15]	Fields were placed into four groups based on mean temperature *minima* over the autumn/winter period.	
Mean $Temp_{max}$ (^{o}C)	<8 [32], 9-9.99 [37] or >10 [33]	Fields were placed into three groups based on mean temperature *maxima* over the autumn/winter period.	See section 4.2.5 for further details meteorological factors
Total rainfall (mm)	0-200 [26], 200-400 [57] or 400+ [19]	Fields were placed into three groups based on total rainfall over the autumn/winter period.	
Mean relative humidity (%)	77.7-82 [22], 82.1-84.9 [41] or 85-88.2 [39]	Fields were placed into three groups based relative humidity over the autumn/winter period.	

4.3 RESULTS

Results presented throughout this chapter are only those that were significant (P≤0.05) in the REML models. Tables 4.4 - 4.7 show the significant (P≤0.05) factors from the REML models for root rot, SBDs and pathogen DNA in soil and *in planta* for GS 21-31, GS 37-45, and GS 65-75. For ease of interpreting results the models were deconstructed to produce summaries for common significant agronomy and meteorological factors. Therefore, tables 4.8 - 4.20 present the significant (P≤0.05) REML predicted means of root rot and SBDs and pathogen DNA quantified in soil and *in planta.*

4.3.1 Agronomy and meteorological factors influencing disease index (DI) of root rot and stem bases diseases and pathogen DNA during crop development

4.3.1.1 Disease index

Table 4.4 summaries the main significant factors and time interactions from the REML model for DI of root rot and SBD's. Time x season interaction was significant for root rot and SBD's suggesting disease development and severity were not consistent across seasons (Table 4.8). Root rot development was also affected by the place of wheat in the rotation, sowing date, $Temp_{max}$ and total rainfall over the autumn/winter period. Factors that affected changes in eyespot index within the crop-growing season were also position of wheat in the rotation, $Temp_{min}$ and $Temp_{max.}$ The only agronomy factor influencing BFR consistently across seasons was sowing date (P<.001). Sharp eyespot development was influenced by soil texture and $Temp_{min.}$

4.3.1.2 Pathogen DNA in soil

Table 4.5 summarises the results of the REML model for pathogen DNA in soil. Time x season interaction was significant for DNA of *O. acuformis* and *M. nivale* indicating that pathogen DNA concentrations in the crop-growing period were not consistent across seasons (Table 4.9). *Rhizoctonia solani* AG 2-1 DNA in soil during crop development was only influenced by previous crop. In contrast, *R.*

cerealis DNA in soil was not affected by any of the agronomy or meteorological factors investigated in this study. Overall the amount of *O. acuformis* DNA in soil was affected by soil texture. DNA of *O. yallundae* in soil was influenced by $Temp_{max}$ and total rainfall. Sowing date and $Temp_{min}$ were highly significant (P<.001) for changes in *M. nivale* DNA in soil. In contrast, $Temp_{max}$ was highly significant for *M. majus* DNA in soil.

4.3.1.3 Roots

Table 4.6 summaries the results of the REML model for pathogen DNA in roots. There were significant changes of AG 2-1, *O. acuformis* and *O. yallundae* between the three growth stages in each season. *Rhizoctonia solani* AG 2-1 and *R. cerealis* DNA in roots showed significant differences between the two seasons but was not affected by any of the agronomy factors investigated in this study. The amount of *O. acuformis* DNA in roots was influenced by soil texture, position of wheat in the rotation, eyespot resistance rating of the current crop, and sowing date. The use of seed treatment also influenced changes in *O. acuformis* DNA concentrations within each season. Position of wheat in the rotation and sowing date significantly affected changes in *O. yallundae* DNA in roots during the crop-growing season. None of the meteorological factors investigated in this study significantly influenced pathogen DNA in roots.

4.3.1.4 Stem bases

Table 4.7 summarises the results of the REML model for pathogen DNA in stem bases. There were time x season interactions for *R. solani* AG 2-1, *R. cerealis*, *O. yallundae* and *M. majus* DNA in stem bases showing changes in the crop-growing period were not consistent across seasons. The time x season interaction was not significant for *O. acuformis* suggesting that changes in DNA for this pathogen were consistent across seasons. Agronomy and meteorological factors influencing AG 2-1 DNA were the position of wheat in the rotation and $Temp_{min.}$ *R. cerealis* was not significantly affected by any of the agronomic or meteorological factors in this study. Concentrations of *O. acuformis* DNA in stem bases were affected by position of wheat in the rotation. $Temp_{max}$ influenced

changes in *O. acuformis* DNA during the crops development. Changes in *O. yallundae* DNA were significantly influenced by the position of wheat in the rotation. Eyespot resistance rating of the crop growing and sowing date also affected *O. yallundae* consistently during the crop development. Previous crop and $Temp_{min}$ affected changes in the amount of *M. nivale* DNA in stem bases between growth stages. *Microdochium majus* DNA in stem bases showed changes between growth stages due to $Temp_{max.}$ Previous crop also influenced *M. majus* DNA in stem bases.

Table 4.4 Agronomy and meteorological factors effecting the DI of root rot, sharp eyespot, eyespot and BFR in English wheat crops in 2011/12 and 2012/13 during crop development (sampled at time GS21-31, GS37-45 and GS65-75) analysed by residual maximum likelihood (REML) analysis (P≤0.05).

	df	Root rot	Eyespot	BFR	Sharp eyespot
Time	2	<.001	<.001	<.001	<.001
Season	1	<.001	<.001	<.001	ns
Soil texture	3	ns	ns	ns	ns
1st 2nd or CW	2	ns	0.013	ns	ns
Sowing date	4	ns	0.004	<.001	ns
$Temp_{max}$ (°C)	2	ns	ns	ns	ns
$Temp_{min}$ (°C)	3	ns	<.001	ns	0.004
Total rainfall	2	ns	ns	ns	ns
Time.Season	2	0.005	<.001	<.001	<.001
Time.Soil texture	6	ns	ns	ns	0.026
Time.1st 2nd or CW	4	0.009	<.001	ns	ns
Time.Sowing date	8	0.004	ns	ns	ns
Time.$Temp_{max}$ (°C)	4	0.005	0.024	ns	ns
Time.$Temp_{min}$ (°C)	6	ns	<.001	ns	<.001
Time.Total rainfall (mm)	4	<.001	ns	ns	ns

df = degrees of freedom
BFR – brown foot rot
Time – samples were collected at GS 21-31, GS 37-45 and GS 65-75
ns – not significant

Table 4.5 Agronomy and meteorological factors effecting pathogen DNA (pg g^{-1} of soil) in soil samples collected from English wheat fields in 2011/12 and 2012/13 at GS21-31, GS37-45 and GS65-75 analysed by residual maximum likelihood (REML) analysis ($P \leq 0.05$).

	df	***R. solani* AG2-1**	***R. cerealis***	***O. acuformis***	***O. yallundae***	***M. nivale***	***M. majus***
Time	2	ns	ns	0.014	ns	0.024	<.001
Season	1	ns	ns	0.006	ns	ns	0.006
Soil texture	3	ns	ns	0.003	0.01	ns	ns
Previous crop	4	0.004	ns	ns	ns	ns	ns
Sowing date	3	ns	ns	ns	ns	<.001	ns
$Temp_{max}$ (°C)	2	ns	ns	ns	0.031	ns	<.001
$Temp_{min}$ (°C)	3	ns	ns	ns	ns	<.001	ns
Total rainfall (mm)	2	ns	ns	ns	ns	ns	ns
Time.Season	2	ns	ns	0.005	ns	0.005	ns
Time.Soil texture	6	ns	ns	ns	ns	ns	ns
Time.Previous crop	8	0.006	ns	ns	ns	ns	ns
Time.Sowing date	6	ns	ns	ns	ns	ns	ns
Time.$Temp_{max}$ (°C)	4	ns	ns	ns	ns	ns	ns
Time.$Temp_{min}$ (°C)	6	ns	ns	ns	ns	ns	ns
Time.Total rainfall (mm)	4	ns	ns	ns	0.003	ns	ns

df = degrees of freedom
Time – samples were collected at GS 21-31, GS 37-45 and GS 65-75
ns – not significant

Table 4.6 Agronomy and meteorological factors effecting pathogen DNA (pg ng^{-1} of total DNA) in roots of wheat plants collected from English wheat fields in 2011/12 and 2012/13 at GS21-31, GS37-45 and GS65-75 analysed by residual maximum likelihood (REML) analysis ($P \leq 0.05$).

	df	***R. solani* AG2-1**	***R. cerealis***	***O. acuformis***	***O. yallundae***
Time	2	<.001	ns	<.001	<.001
Season	1	ns	<.001	0.022	ns
Soil texture	3	ns	ns	0.024	ns
1st 2nd or CW	2	ns	ns	0.001	0.006
Eyespot rating	3	ns	ns	<.001	ns
Sowing date	4	ns	ns	<.001	0.001
Seed treatment	5	ns	ns	<.001	ns
Time.Season	4	0.001	ns	0.008	<.001
Time.Soil texture	2	ns	ns	ns	ns
Time.1st 2nd or CW	4	ns	ns	ns	0.018
Time.Eyespot rating	3	ns	ns	ns	ns
Time.Sowing date	8	ns	ns	ns	0.004
Time.Seed treatment	10	ns	ns	<.001	ns

df = degrees of freedom
Time – samples were collected at GS 21-31, GS 37-45 and GS 65-75
ns – not significant

Table 4.7 Agronomy and meteorological factors effecting pathogen DNA (pg ng^{-1} of total DNA) in stem bases of wheat plants collected from English wheat fields in 2011/12 and 2012/13 at GS21-31, GS37-45 and GS65-75 analysed by residual maximum likelihood (REML) analysis ($P \leq 0.05$).

	df	***R. solani* AG2-1**	***R. cerealis***	***O. acuformis***	***O. yallundae***	***M. nivale***	***M. majus***
Time	2	<.001	ns	<.001	<.001	ns	<.001
Season	1	0.003	<.001	<.001	<.001	<.001	0.012
Previous crop	4	ns	ns	ns	ns	0.014	0.011
1st 2nd or CW	2	0.006	ns	0.025	<.001	ns	ns
Eyespot rating	3	ns	ns	ns	<.001	ns	ns
Sowing date	4	ns	ns	ns	<.001	ns	ns
$Temp_{max}$ (^{o}C)	2	ns	ns	0.03	ns	ns	0.002
$Temp_{min}$ (^{o}C)	3	0.003	ns	ns	ns	0.01	ns
Relative humidity (%)	2	ns	ns	ns	ns	ns	0.006
Time.Season	2	0.031	0.009	ns	<.001	<.001	0.015
Time.Previous crop	8	ns	ns	ns	ns	0.025	ns
Time.1st 2nd or CW	4	ns	ns	ns	0.005	ns	ns
Time.Eyespot rating	6	ns	ns	ns	ns	ns	ns
Time.Sowing date	8	ns	ns	ns	ns	ns	ns
Time.$Temp_{max}$ (^{o}C)	4	ns	ns	0.008	ns	ns	0.01
Time.$Temp_{min}$ (^{o}C)	6	ns	ns	ns	ns	0.009	ns
Time.Relative humidity	6	ns	ns	ns	ns	ns	ns

df = degrees of freedom
Time – samples were collected at GS 21-31, GS 37-45 and GS 65-75
ns – not significant

4.3.2 Development of root rot and stem base diseases

Differences for root rot and SBD development between and within seasons were detected by REML showing significant time x season interaction (Table 4.8).

Root rot index was significantly higher in 2011/12 compared to 2012/13 (Table 4.8). In both seasons root rot declined over the growing season. However, the rate of decline was not consistent in both seasons with root rot declining faster in 2011/12 (15 to 5.3%) than 2012/13 (11.9 to 1.7%).

Eyespot index was higher in 2011/12 than 2012/13; this was most notable at GS 37-45 and GS 65-75 (Table 4.8). The pattern of development in 2011/12 showed eyespot significantly increased from GS 21-31 (5.9%) to GS 37-45 (37%) but then declined at GS 65-75 (29.6%). In the following season eyespot was low and showed only marginal increases over the season.

BFR index was significantly lower at GS 21-31 and GS 37-45 in 2011/12 than at the same growth stages in 2012/13 (Table 4.8). However, at GS 65-75 in both seasons BFR was found at comparable levels. Changes in BFR index over time showed significant increases between GS 21-31 and GS 37-45 in both seasons.

Sharp eyespot index was higher in 2011/12 than 2012/13 (Table 4.8). In 2011/12 at GS 21-31 sharp eyespot was not detected but in 2012/13 was found at low levels (4.7%). The pattern of development was not consistent in both seasons. Sharp eyespot in 2011/12 increased at each successive growth stage from 0% (GS21-31) to 17% (GS 65-75). However, in the following season the disease failed to develop and remained low throughout the period of study.

Table 4.8 Effect of season (2011/12 and 2012/13) on root and stem diseases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

Season*	**2011/12 [52]**	**2012/13 [50]**	**SED[a]**	**SED[b]**	**SED[c]**
Disease[1]					
Time.Root rot					
T1	15.0	11.9	1.5	1.6	1.4
T2	12.9	6.7			
T3	5.3	1.7			
Time.Eyespot					
T1	5.9	5.5	3.3	3.6	2.8
T2	37.0	9.9			
T3	29.6	9.8			
Time.BFR					
T1	12.9	21.8	1.6	1.5	1.5
T2	26.2	29.7			
T3	29.1	27.1			
Time.Sharp eyespot					
T1	0.00	4.7	1.8	1.8	1.7
T2	11.2	8.1			
T3	17.0	5.1			

* - Only significant ($P \leq 0.05$) effects on pathogen and/or diseases are presented. SED[a] for comparing means between all factor levels. SED[b] for comparing means between growth stages within the same season. SED[c] for comparing means between seasons. [-] - Number of observations per factor level. [1] – Disease expressed as disease index (%). T1 – GS 21-31 (n = 102), T2 – GS 37-45 (n = 102), T3 – GS 65-75 (n -102).

4.3.3 Seasonal effects on pathogen DNA accumulation in soil, roots and stem bases

The amount of AG 2-1 DNA in roots and stem bases was relatively low but was affected by season and showed changes between growth stages (Table 4.9 and 4.10). Changes between growth stages showed AG 2-1 DNA in roots remained unchanged during 2011/12 but in 2012/13 showed a small increasing trend. In stem bases AG 2-1 DNA showed an increasing trend in both seasons.

Rhizoctonia cerealis DNA concentrations were considerably higher in stem bases than roots (Table 4.9 and 4.10). In roots DNA was 6 fold higher in 2011/12 than 2012/13. In stem bases *R. cerealis* DNA was >22 fold higher at each growth stage in 2011/12 than in 2012/13. The highest DNA concentrations occurred in 2011/12 at GS 21-31 (0.62 pg ng^{-1}). The time x season interaction showed that in 2011/12 *R. cerealis* DNA declined at each successive but in the following season an increasing trend was found.

Season effected *O. acuformis* DNA concentrations in soil, roots and stem bases (Table 4.9 and 4.10). In the soil *O. acuformis* DNA was higher at each growth stage in 2012/13 than 2011/12. The pattern of development in soil showed a declining trend in 2011/12 but increased at each successive growth stage in 2012/13. In roots in both seasons *O. acuformis* DNA increased at each successive growth stage. Overall in stem bases *O. acuformis* DNA was higher in 2011/12 (1.08 pg ng-1) than 2012/13 (0.21 pg ng^{-1}).

Oculimacula yallundae DNA was found at higher levels in stem bases than in roots (Table 4.9 and 4.10). *O. yallundae* DNA concentrations in roots were higher in 2011/12 than 2012/13 but showed an increasing trend in both seasons. *O. yallundae* DNA in stems bases also increased (>80 fold) in both seasons. Higher DNA levels were shown at GS 21-31 and GS 37-45 in 2011/12 than in 2012/13 but by GS 65-75 they were at similar levels.

DNA concentrations of *M. nivale* in soil and stem bases were effected by season (Table 4.9 and 4.10). In 2011/12 the highest *M. nivale* DNA levels in soil occurred at GS 21-31 then declined over the season. In the following season DNA was low and remained relatively unchanged. In stem bases *M. nivale* DNA was high at GS 21-31 (5.13 pg ng^{-1}) in 2011/12 then declined 21 fold over the season. In 2012/13 DNA was lower but increased 3.4 fold over the season.

Microdochium majus DNA in soil and stem bases also showed seasonal changes (Table 4.9 and 4.10). DNA concentrations of *M. majus* in soil were on average 11 fold higher in 2012/13 than in 2011/12 (Table 4.6). *Microdochium majus* DNA in stem bases was higher at each growth stage in 2011/12 than 2012/13. In both seasons *M. majus* DNA showed the same increasing trend over the season.

Table 4.9 Effect of season (2011/12 and 2012/13) on pathogen DNA in soil and roots. Values are REML-predicted means from wheat crops sampled T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

Season*	**2011/12 [52]**		**2012/13 [50]**		**SED[a]**	**SED[b]**	**SED[c]**
Soil[1]							
O. acuformis							
T1	-1.359	(0.044)	-0.944	(0.114)	0.303	0.346	0.227
T2	-2.281	(0.005)	-0.883	(0.131)			
T3	-1.543	(0.029)	-0.799	(0.159)			
M. majus	-3.263	(0.001)	-1.960	(0.011)	0.428		
M. nivale							
T1	-0.565	(0.272)	-2.464	(0.003)	0.464	0.502	0.408
T2	-2.294	(0.005)	-2.276	(0.005)			
T3	-1.449	(0.036)	-2.331	(0.005)			
Roots[2]							
R. cerealis AG 2-1	-1.933	(0.012)	-2.809	(0.002)	0.200		
T1	-3.791	(0.0002)	-3.979	(0.0001)	0.089	0.092	0.082
T2	-3.767	(0.0002)	-3.612	(0.0002)			
T3	-3.743	(0.0002)	-3.506	(0.0003)			
O. acuformis							
T1	-3.165	(0.001)	-2.483	(0.003)	0.275	0.204	0.293
T2	-1.720	(0.019)	-1.881	(0.013)			
T3	-1.132	(0.074)	-0.946	(0.113)			
O. yallundae							
T1	-2.827	(0.001)	-2.386	(0.004)	0.252	0.227	0.249
T2	-0.913	(0.122)	-1.947	(0.011)			
T3	-0.502	(0.315)	-0.094	(0.805)			

* - Only significant ($P \leq 0.05$) effects on pathogen and/or diseases are presented. SED[a] for comparing means between all factor levels. SED[b] for comparing means between growth stages within the same season. SED[c] for comparing means between seasons. [-] - Number of observations per factor level. [1] – Pathogen DNA in soil expressed as pg g^{-1} of soil, [2] – pathogen DNA in *planta* expressed as pg ng^{-1} of total DNA. T1 –GS 21-31 (n = 102), T2 – GS 37-45 (n = 102), T3 – GS 65-75 (n -102).

Table 4.10 Effect of season (2011/12 and 2012/13) on pathogen DNA in stem bases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

Season*	**2011/12 [52]**		**2012/13 [50]**		**SED[a]**	**SED[b]**	**SED[c]**
Stem bases[1]							
AG 2-1							
T1	-3.763	(0.0002)	-4.009	(0.0001)	0.098	0.094	0.097
T2	-3.864	(0.0001)	-3.911	(0.0001)			
T3	-3.432	(0.0004)	-3.783	(0.0002)			
R. cerealis							
T1	-0.209	(0.618)	-2.654	(0.002)	0.262	0.278	0.237
T2	-0.329	(0.469)	-2.520	(0.003)			
T3	-0.473	(0.337)	-1.838	(0.015)			
O. acuformis	0.034	(1.082)	-0.678	(0.210)	0.167		
O. yallundae							
T1	-0.926	(0.119)	-2.038	(0.009)	0.240	0.224	0.227
T2	0.045	(1.11)	-0.909	(0.123)			
T3	0.983	(9.62)	0.914	(8.19)			
M. nivale							
T1	0.710	(5.129)	-0.712	(0.194)	0.251	0.248	0.247
T2	-0.411	(0.388)	-0.354	(0.443)			
T3	-0.614	(0.243)	-0.178	(0.664)			
M. majus							
T1	-0.100	(0.794)	-1.698	(0.020)	0.298	0.334	0.266
T2	-0.417	(0.383)	-0.732	(0.186)			
T3	0.213	(1.635)	-0.154	(0.701)			

* - Only significant ($P \leq 0.05$) effects on pathogen and/or diseases are presented. SED[a] for comparing means between all factor levels. SED[b] for comparing means between growth stages within the same season. SED[c] for comparing means between seasons. [-] - Number of observations per factor level. [1] – pathogen DNA in *planta* expressed as pg ng^{-1} of total DNA. T1 – GS 21-31 (n = 102), T2 – GS 37-45 (n = 102), T3 – GS 65-75 (n -102).

4.3.4 Influence of agronomy factors on individual diseases and pathogens throughout crop development

4.3.4.1 Soil texture

Sharp eyespot severity showed an increasing trend between growth stages regardless of soil texture (Table 4.11). However, in silty clay loam soils sharp eyespot developed slower and was less severe than in sandy clay loam, clay/clay loam and sandy loam.

O. acuformis and *O. yallundae* DNA concentrations in soil were influenced by soil texture (Table 4.11). The amount of DNA was 63 and 95.6 higher in silty clay loam soils than sandy loam soils for *O. acuformis* and *O. yallundae* respectively. *O. acuformis* in roots was also significantly lower when the crop was grown in silty clay loam soils compared to the other three soil textures.

4.3.4.2 Previous crop

In soil the only pathogen influenced by previous crop was *R. solani* AG 2-1 (Table 4.12). The highest AG 2-1 DNA concentrations in soil occurred when the previous crop was OSR and the lowest in continuous wheat fields. DNA concentrations of AG 2-1 after OSR were 2560, 2990 and 104-fold higher at GS 21-31, GS 37-45 and GS 65-75, respectively, than at the same growth stages in continuous wheat fields. There were also significantly higher levels of AG 2-1 DNA in soil after winter wheat at each growth stage than in continuous wheat fields. The only significant change in AG 2-1 DNA in soil between growth stages occurred when legume was a previous crop when the amount of DNA declined 166.5 fold between GS 21-31 and GS 37-45. However, trends were shown with AG 2-1 declining 6.2 and 4.5 fold between GS 21-31 and GS 65-75 when the previous crop was OSR and winter wheat respectively.

DNA levels of *M. nivale* and *M. majus* in stem bases was also affected by previous crop (Table 4.12). Overall *M. majus* DNA in stem bases was highest when the previous crop was a legume and lowest when it was 'other' (oats/maize). Similar amounts of DNA in stem bases were found after OSR and wheat. The highest *M. nivale* DNA in stem bases also occurred after legume (peas/beans); DNA concentrations were notably high (7.03 pg g^{-1} of soil) at GS 21-31. The development of *M. nivale* DNA in stem bases showed contrasting trends depending on the previous crop. When the previous crop was a legume DNA significantly declined 26.7 fold between GS 21-31 and GS 37-45 then increased 7.1 fold at GS 65-75. In continuous wheat fields *M. nivale* DNA declined 23 fold over the season. There were no significant changes in the amount of *M. nivale* DNA in stem bases when previous crops were OSR, winter wheat or other (oats/maize).

.

Table 4.11 Effect of soil texture on pathogen DNA in soil and roots and disease. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

Soil texture*	**Silty clay loam [11]**		**Sandy clay loam [52]**		**Clay/clay loam [24]**		**Sandy loam [15]**		**SED[a]**	**SED[b]**	**SED[c]**
Disease[1]											
Time.Sharp eyespot											
T1	2.6		2.2		2.3		1.8		2.2	2.2	1.4
T2	5.3		10.6		10.9		11.8				
T3	8.5		12.3		12.7		10.5				
Soil [2]											
O. acuformis	-2.262	(0.005)	-0.813	(0.154)	-1.629	(0.023)	-0.500	(0.316)	0.491		
O. yallundae	-2.488	(0.003)	-0.969	(0.107)	-0.979	(0.105)	-0.542	(0.287)	0.526		
Roots[3]											
O. acuformis	-2.223	(0.006)	-1.624	(0.024)	-1.909	(0.012)	-1.795	(0.016)	0.194		

* - Only significant (P≤0.05) effects on pathogen and/or diseases are presented. SED[a] for comparing means between all factor levels, SED[b] for comparing means between growth stages within the same level of soil texture. SED[c] for comparing means between soil textures. [-] - Number of observations per factor level. T1 – GS 21-31, T2 – GS 37-45, T3 – GS 65-75. [1] – Disease is expressed as disease index (%). [2] – Pathogen DNA in soil expressed as pg g^{-1} of soil. [3] – pathogen DNA in *planta* expressed as pg ng^{-1} of total DNA.

Table 4.12 Effect of previous crop on pathogen DNA in soil and stem bases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

Previous crop*	**OSR [43]**		**WW [33]**		**Legume [6]**		**Other [10]**		**CW [10]**		**SED[a]**	**SED[b]**	**SED[c]**
Soil[1]													
Time.*R. solani* AG 2-1													
T1	0.408	(2.56)	-0.362	(0.435)	-0.477	(0.333)	-2.054	(0.009)	-3.218	(0.001)	1.043	1.118	0.594
T2	0.475	(2.99)	-0.261	(0.550)	-2.821	(0.002)	-1.448	(0.036)	-3.111	(0.001)			
T3	-0.381	(0.42)	0.297	(1.98)	-1.083	(0.083)	-1.492	(0.032)	-2.389	(0.004)			
Stem bases[2]													
M. majus	-0.552	(0.281)	-0.673	(0.212)	0.034	(1.08)	-1.021	(0.095)	-0.195	(0.639)	0.274		
Time.*M. nivale*													
T1	-0.027	(0.940)	-0.251	(0.561)	0.847	(7.03)	0.039	(1.094)	-0.614	(0.243)	0.370	0.366	0.350
T2	-0.167	(0.680)	-0.040	(0.911)	-0.579	(0.263)	-0.552	(0.281)	-0.574	(0.267)			
T3	-0.174	(0.671)	-0.174	(0.670)	0.271	(1.87)	-1.332	(0.047)	-0.571	(0.269)			

* - Only significant ($P \leq 0.05$) effects on pathogen and/or diseases are presented. SED[a] for comparing means between all factor levels, SED[b] for comparing means between growth stages within the same level of previous crop. SED[c] for comparing means between previous crops. [-] - Number of observations per factor level. [1] – Pathogen DNA in soil expressed as pg g^{-1} of soil. [2] – pathogen DNA in *planta* expressed as pg ng^{-1} of total DNA. OSR – oilseed rape, WW-winter wheat, legume (peas or beans), other – maize and oats, and CW continuous wheat. T1 – GS 21-31 (n = 102), T2 – GS 37-45 (n = 102), T3 – GS 65-75 (n -102).

4.3.4.3 Position of wheat in the rotation

Root rot index was highest at GS 21-31 but showed a declining trend in 1st, 2nd and continuous wheats (CW) over the seasons (Table 4.13). Although the differences in root rot index was small regardless of the position of wheat in the rotation, at GS 65-75 root rot was significantly higher in 1st wheats (4.8%) than CW (2.1%).

In stem bases AG 2-1 DNA concentrations were low overall but were found at higher levels in 1st and CW than 2nd wheats (Table 4.13).

Regardless of the position of wheat in the rotation eyespot index was significantly higher at GS 65-75 than GS 21-31 (Table 4.13). However, the pattern of development was not the same in 1st, 2nd and CWs. In 1st wheats eyespot index increased between GS 21-31 (5.6%) and GS 37-45 (21%) but then significantly declined at GS 65-75 (13.3%). The pattern of development in 2nd and CWs showed eyespot also increased between GS 21-31 and GS 37-45 but then remained relatively unchanged at GS 65-75. In roots overall *O. acuformis* DNA was 3.2 and 3.3 fold higher in 2nd and continuous wheats respectively than in 1st wheat crops (Table 4.13). The amount of *O. yallundae* DNA in roots increased at each successive growth stage regardless of the position of wheat in the rotation (Table 4.13). However, by GS 65-75 DNA concentrations were 6.2 and 14.3 fold higher in 2nd and CW than 1st wheats respectively. In stem bases *O. acuformis* DNA was significantly higher in CW than in 1st wheat crops. *Oculimacula yallundae* DNA concentrations in stem bases also increased at each successive growth stage. Even so the largest increases were shown in 2nd wheats where DNA levels increased 913 fold between GS 21-31 and GS 65-75. In addition, the amount of *O. yallundae* DNA at GS 65-75 was also considerably higher in 2nd wheats (24.7 pg ng^{-1}) than 1st (5.1pg ng^{-1}) and CWs (5.5 pg ng^{-1}).

4.3.4.4 Sowing date

Root rot showed a declining trend over the season regardless of sowing date. The declining trend was greater in early (1^{st}-14^{th} Sept) than late (November) sown crops (Table 4.14). Root rot at GS 21-31 was significantly higher in crops sown from 1^{st} to 14^{th} of September than those in November. In contrast at GS 65-75 root rot was higher in late (November) than early sown crops (1^{st}-14^{th} Sept).

Eyespot index was significantly higher (22.4%) in early (1^{st}-14^{th} Sept) sown crops than those in November (7.5%) (Table 4.14). Overall *O. acuformis* DNA levels in roots of crops sown in November were significantly lower than those at all other sowing dates (Table 4.14). DNA concentrations of *O. yallundae* in roots throughout the season were also lowest in November sown crops compared to all other sowing dates (Table 4.14). That said *O. yallundae* DNA concentrations in roots regardless of sowing date increased at each successive growth stage. The largest increase was 940 fold between GS 21-31 and GS 65-75 in 1^{st}-14^{th} October sowing dates. The highest *O. yallundae* DNA concentrations in roots occurred in early sown crops (1^{st}-14^{th} Sept) at GS 65-75 (1.54 pg ng^{-1}). In stem bases *O. yallundae* DNA was significantly higher in crops sown in September and October compared to November (Table 4.14).

BFR index was also significantly higher at 28.6% in early (1^{st}-14^{th} Sept) compared to late sown crops at 17.2% (November) (Table 4.14). The same pattern was also shown for *M. nivale* in soil with DNA levels 42 fold higher in early (1^{st}-14^{th} Sept) than in later sown crops (November).

Table 4.13 Effect of position of wheat in the rotation (1st, 2nd or continuous wheat (CW)) on pathogen DNA in roots and stem bases and diseases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

	1st wheat [59]		2nd wheat [33]		CW [10]		SED[a]	SED[b]	SED[c]
Disease[1]									
Time.Root rot									
T1	12.2		13.4		14.7		1.4	1.3	1.3
T2	9.5		9.1		10.7				
T3	4.8		3.6		2.1				
Time.Eyespot									
T1	5.6		5.5		6.0		3.1	3.1	2.7
T2	21.0		25.6		23.9				
T3	13.3		22.4		23.4				
Roots[2]									
O. acuformis	-2.234	(0.006)	-1.728	(0.019)	-1.702	(0.020)	0.182		
Time.*O. yallundae*									
T1	-2.8	(0.002)	-2.524	(0.003)	-2.497	(0.003)	0.335	0.336	0.305
T2	-1.627	(0.024)	-0.913	(0.122)	-1.749	(0.018)			
T3	-0.946	(0.113)	-0.158	(0.695)	0.209	(1.618)			
Stem bases[2]									
R. solani AG 2-1	-3.712	(0.0002)	-3.848	(0.0001)	-3.821	(0.0002)	0.069		
O. acuformis	-1.174	(0.067)	-0.807	(0.156)	-0.699	(0.200)	0.228		
Time.O. yallundae									
T1	-1.882	(0.013)	-1.565	(0.027)	-1.000	(0.100)	0.321	0.325	0.277
T2	-1.027	(0.094)	-0.181	(0.659)	-0.087	(0.818)			
T3	0.710	(5.126)	1.392	(24.7)	0.743	(5.54)			

* - Only significant (P≤0.05) effects on pathogen and/or diseases are presented. SED[a] for comparing means between all factor levels, SED[b] for comparing means between growth stages within the same level of position of wheat in the rotation. SED[c] for comparing means between 1st, 2nd or CW. [-] - Number of observations per factor level. [1] – Disease is expressed as disease index (%). [2] – pathogen DNA in *planta* expressed as pg ng^{-1} of total DNA. (%). T1 – GS 21-31, T2 – GS 37-45, T3 – GS 65-75.

Table 4.14 Effect of sowing date on pathogen DNA in soil, roots and stem bases and diseases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

Sowing date[*]	**1st-14th Sept** [*n* = 18]		**15th-30th Sept** [*n* = 22]		**1st-14th Oct** [*n* = 40]		**15th-31st Oct** [*n* = 15]		**Nov** [*n* = 7]		**SED**[a]	**SED**[b]	**SED**[c]
Disease[1]													
Time.Root rot													
T1	15.4		14.7		14.9		13.9		8.2		1.7	1.62	1.60
T2	9.91		11.4		10.5		7.27		9.8				
T3	1.8		4.0		3.3		3.5		4.8				
Eyespot	22.4		16.0		19.2		16.4		7.5		2.9		
BFR	28.6		26.2		26.4		24.0		17.2		1.8		
Soil[2]													
M. nivale	-1.374	(0.042)	-2.29	(0.005)	-1.573	(0.027)	-2.284	(0.005)	-2.887	(0.001)	0.471		
Roots[3]													
O. acuformis	-1.516	(0.030)	-1.711	(0.01)	-1.960	(0.011)	-1.853	(0.014)	-2.40	(0.004)	0.194		
Time.*O. yallundae*													
T1	-1.848	(0.014)	-2.094	(0.008)	-2.852	(0.001)	-2.761	(0.002)	-3.48	(0.0003)	0.403	0.396	0.37
T2	-1.234	(0.058)	-1.413	(0.039)	-1.390	(0.041)	-1.289	(0.051)	-1.82	(0.015)			
T3	0.186	(1.54)	-0.277	(0.53)	-0.026	(0.94)	0.055	(1.14)	-1.43	(0.04)			
Stem bases[3]													
O. yallundae	0.222	(1.67)	-0.206	(0.62)	-0.134	(0.734)	-0.314	(0.49)	-1.18	(0.07)	0.283		

* - Only significant ($P \leq 0.05$) effects on pathogen and/or diseases are presented. SED[a] for comparing means between all factor levels, SED[b] for comparing means between growth stages within the same level of sowing date. SED[c] for comparing means between sowing dates. [-] - Number of observations per factor level. [1] – disease expressed as disease index (%). [2] – Pathogen DNA in soil expressed as pg g^{-1} of soil. [3] – pathogen DNA in *planta* expressed as pg ng^{-1} of total DNA. T1 –GS 21-31, T2 – GS 37-45, T3 – GS 65-75.

4.3.4.5 Eyespot resistance rating of the growing crop

Eyespot resistance rating of the growing crop effected concentrations of *O. acuformis* DNA in roots and *O. yallundae* DNA in stem bases (Table 4.15). There were significantly higher *O. acuformis* DNA in roots when varieties grown had resistance ratings of four and five compared to ratings of six and eight. In stem bases *O. yallundae* DNA was 2.8, 7.1 and 7.4 fold lower in varieties with resistance ratings of four, five and six compared to ratings of eight respectively.

4.3.4.6 Seed treatment

Seed treatment affected the development of *O. acuformis* DNA in roots (Table 4.16). Differences were shown at GS 37-45 when the highest concentrations of *O. acuformis* DNA in roots were in silthiofam treatments and the lowest fludioxonil + silthiofam. At GS 65-75 *O. acuformis* DNA in roots from silthiofam treatments was significantly higher than all other seed treatments. The development of *O. acuformis* DNA in roots in all seed treatments showed a significant increasing trend from GS 21-31 to GS 65-75. The largest change detected was from silthiofam seed treatments were *O. acuformis* DNA increased 1920-fold. The smallest change was found in fludioxonil + silthiofam were *O. acuformis* DNA increased 16.2-fold.

Table 4.15 Effect of eyespot resistance rating (ER) on pathogen DNA in roots and stem bases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

Eyespot resistance rating*	**ER-4[2] [26]**		**ER-5 [45]**		**ER-6 [20]**		**ER-8 [11]**		**SED**
Roots[1]									
O. acuformis	-2.15	(0.007)	-2.13	(0.007)	-1.52	(0.030)	-1.74	(0.018)	0.168
Stem bases[1]									
O. yallundae	-0.363	(0.434)	-0.029	(0.935)	-0.013	(0.970)	-0.882	(0.131)	0.245

* - Only significant (P≤0.05) effects on pathogen and/or diseases are presented. [-] - Number of observations per factor level. [1] – Pathogen DNA in *planta* expressed as pg ng^{-1} of total DNA. T1 –GS 21-31, T2 – GS 37-45, T3 – GS 65-75. [2] - Disease rating on the 1-9 scale, high figure indicates variety has a high degree of resistance.

Table 4.16 Effect of seed treatments on pathogen DNA in roots. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

Seed treatment*	**Prothioconazole [27]**		**Prothioconazole + clothianidin [43]**		**Prothioconazole + clothianidin + silthiofam [12]**		**Silthiofam [5]**		**Fludioxonil + silthiofam [9]**		**Other [6]**	
Roots[1]												
Time.*O. acuformis*												
T1	-2.203	(0.006)	-2.467	(0.003)	-2.799	(0.002)	-3.744	(0.0002)	-2.321	(0.005)	-3.410	(0.0004)
T2	-1.602	(0.025)	-1.865	(0.014)	-2.093	(0.008)	-1.081	(0.083)	-2.605	(0.002)	-1.555	(0.028)
T3	-0.754	(0.176)	-1.276	(0.053)	-1.440	(0.036)	-0.416	(0.384)	-1.092	(0.081)	-1.256	(0.055)

	SED[a]	**SED**[b]	**SED**[c]
Seed treatment	0.462	0.437	0.466

* - Only significant (P≤0.05) effects on pathogen and/or diseases are presented. SED[a] for comparing means between all factor levels, SED[b] for comparing means between growth stages within the same level of seed treatment. SED[c] for comparing means between the seed treatments. [-] - Number of observations per factor level. [1] – Pathogen DNA *in planta* expressed as pg ng^{-1} of total DNA. T1 –GS 21-31 (n = 102), T2 – GS 37-45 (n = 102), T3 – GS 65-75 (n -102).

Details of seed treatments - active ingredients and products names in parenthesis. Prothioconazole 100 g a.i l^{-1} (Redigo), Prothioconazole 50 g a.i l^{-1} + clothianidin 250 g a.i l^{-1} (Redigo Deter), Prothioconazole + clothianidin & silthiofam 125 g a.i l^{-1} (Redigo Deter + Latitude). Silthiofam 125 g a.i l^{-1} (Latitude), Fludioxonil 25 g a.i l^{-1} & silthiofam 125 g a.i l^{-1} (Beret Gold + Latitude), 'Other' = Prothioconazole 50 g a.i l^{-1} + clothianidin 250 g a.i l^{-1} + fluquinconazole 167 g a.i l^{-1} (Redigo Deter + Galmano). Fludioxonil 25 g a.i l^{-1} + difenoconazole 25 g a.i l^{-1} (Celest extra).

4.3.5 Influence of environmental factors on individual disease and pathogens throughout crop development

4.3.5.1 Temperature

Root rot index showed a declining trend over the season regardless of the mean $Temp_{max}$ over the autumn/winter period (Table 4.17). However, at GS 65-75 root rot was significantly lower where the $Temp_{max}$ over the autumn/winter period had been >10°C. Therefore, the decline in root rot was greatest in areas with the warmest autumn/winter period.

Sharp eyespot at GS 21-31 was not detected in areas where the autumn/winter period was <2.99°C and was only found at very low levels in the other $Temp_{min}$ ranges (Table 4.18). The development of sharp eyespot between GS 21-31 and GS 37-45 was marginal in the lowest $Temp_{min}$ (<2.99°C) but then increased significantly at GS 65-75. In contrast sharp eyespot in the highest $Temp_{min}$ (>5°C) increased considerably by GS 37-45 (16.7%) but then declined at GS 65-75 (12.7%).

Eyespot index was influenced by $Temp_{max}$ and $Temp_{min}$ (Table 4.17 and 4.18). Eyespot index was significantly higher at GS 65-75 compared to GS 21-31 regardless of the mean $Temp_{max}$ and $Temp_{min}$ over the autumn/winter. The main difference occurred at GS 21-31 when eyespot index was higher in areas that had the warmest autumn/winter period. The pattern of development over the season was generally similar with eyespot index peaking at GS 37-45 before declining at GS 65-75. Overall the highest *O. yallundae* DNA concentrations in soil occurred in areas were the autumn/winter $Temp_{max}$ was <8.99°C; these were 13.3 and 15.1 fold higher than at 9-9.99 and >10°C, respectively (Table 4.17). DNA concentrations of *O. acuformis* in stem bases showed an increasing trend over the season regardless of the mean $Temp_{max}$ over the autumn/winter period (Table 4.17). The highest *O. acuformis* DNA levels at each growth stage occurred in areas were the $Temp_{max}$ had been <8.99°C. This was most notable at GS 65-75 when *O. acuformis* DNA was 8.5 and 34.7 fold higher at <8.99°C than at 9-9.99 and >10°C respectively.

R. solani AG 2-1 DNA concentrations were significantly higher when the mean $Temp_{min}$ over the autumn/winter was <2.99°C than the other $Temp_{min}$ ranges (Table 4.18).

Microdochium majus was influenced by $Temp_{max}$ over the autumn/winter period (Table 4.17) and *M. nivale* by $Temp_{min}$ in the same period (Table 4.18). *M. majus* DNA in soil was 11 fold higher in areas were the autumn/winter $Temp_{max}$ was <8.99°C compared to 9-9.99 and >10°C. DNA concentrations of *M. majus* in stem bases showed an increasing trend at each growth stage regardless of the mean $Temp_{max}$ over the autumn/winter period. At each growth stage *M. majus* DNA levels were higher at the lowest $Temp_{max}$ (<8.99°C) than the other two warmer $Temp_{max}$ ranges. The highest *M. nivale* DNA levels in soil occurred in areas were the mean autumn/winter $Temp_{min}$ was <2.99°C; these were 849 fold higher than at >5°C. *M. nivale* DNA levels in stem bases were also higher at GS 21-31 when the $Temp_{min}$ was <2.99°C but then significantly declined 46 fold by GS 65-75. In contrast in the other $Temp_{min}$ ranges *M. nivale* DNA showed no significant changes.

4.3.5.2 Total rainfall

Root rot index showed a declining trend regardless of the total rainfall over the autumn/winter period (Table 4.19). The main difference at GS 21-31 was the higher root rot index (16.4%) when total rainfall was >400mm compared to 200-399 (13.6%) and <199mm (10.2%). However, by GS 65-75 there was no difference between root rot in any of the total rainfall ranges.

In soil *O. yallundae* DNA concentrations at GS 21-31 was quantified at highest levels in the lowest total rainfall (<199 mm) range (Table 4.19). These were 21.4 and 520 fold higher than in areas were rainfall was 200-399 and >400 mm respectively. The only significant changes in *O. yallundae* DNA in soil between the growth stages occurred in the lowest rainfall range (<199 mm) when DNA decreased 104 fold between GS 21-31 and GS 37-45 and increased 38.7 fold GS 65-75.

4.3.5.3 Relative humidity

The main pathogen to be influenced by relative humidity was *M. majus* DNA in stem bases (Table 4.20). Overall the amount of *M. majus* DNA in stem bases was 2.1 fold higher in areas were the relative humidity over the autumn/winter was <84.9% compared to >85%.

Table 4.17 Effect of mean $Temp_{max}$ (°C) over the autumn/winter period on pathogen DNA in soil and stem bases and disease. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

$Temp_{max}$ (°C)*	<8.99 [32]		9-9.99 [37]		>10 [33]		SED[a]	SED[b]	SED[c]
Disease[1]									
Root rot									
T1	12.4		13.6		14.3		1.5	1.5	1.4
T2	11.5		8.5		9.3				
T3	5.7		4.1		0.7				
Eyespot									
T1	3.2		4.5		9.5		3.6	3.6	3.1
T2	26.7		24.1		19.6				
T3	20.9		22.5		15.7				
Soil[2]									
O. yallundae	-0.479	(0.332)	-1.604	(0.025)	-1.650	(0.022)	0.437		
M. majus	-1.969	(0.011)	-2.919	(0.001)	-2.947	(0.001)	0.430		
Stem bases[3]									
O. acuformis									
T1	-1.389	(0.041)	-1.499	(0.032)	-1.634	(0.023)	0.305	0.323	0.253
T2	-0.726	(0.188)	-0.987	(0.103)	-1.935	(0.012)			
T3	0.866	(7.35)	-0.062	(0.867)	-0.674	(0.212)			
M. majus									
T1	-0.554	(0.280)	-0.782	(0.165)	-1.361	(0.044)	0.430	0.317	0.283
T2	-0.158	(0.694)	-0.630	(0.235)	-0.935	(0.116)			
T3	0.458	(2.87)	-0.038	(0.917)	-0.332	(0.466)			

* - Only significant ($P \leq 0.05$) effects on pathogen and/or diseases are presented. SED[a] for comparing means between all factor levels, SED[b] for comparing means between growth stages within the same level of $Temp_{max.}$ SED[c] for comparing means between $Temp_{max}$ ranges. [-] - Number of observations per factor level. [1] – Disease expressed as disease index (%). [2] – Pathogen DNA in soil expressed as pg g^{-1} of soil. [3] – pathogen DNA in *planta* expressed as pg ng^{-1} of total DNA. T1 – GS 21-31 (n = 102), T2 – GS 37-45 (n = 102), T3 – GS 65-75 (n -102).

Table 4.18 Effect of mean $Temp_{min}$ (°C) over the autumn/winter period on pathogen DNA in soil and stem bases and diseases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

$Temp_{min}$ (°C)*	<2.9 [9]		3-3.99 [41]		4-4.9 [37]		5> [15]		SED[a]	SED[b]	SED[c]
Disease[3]											
Eyespot											
T1	5.0		4.1		4.3		9.5		4.0	4.1	3.4
T2	16.3		20.9		28.3		28.4				
T3	16.2		23.3		19.8		19.6				
Sharp eyespot											
T1	0.0		3.4		3.8		3.3		2.5	2.5	2.4
T2	3.9		8.7		9.2		16.7				
T3	10.2		9.9		11.2		12.7				
Soil[1]											
M. nivale	-0.071	(0.849)	-1.649	(0.022)	-2.206	(0.006)	-2.843	(0.001)	0.495		
Stem bases[2]											
Time.*R. solani* AG 2-1	-3.526	(0.0003)	-3.776	(0.0002)	-3.891	(0.0001)	-3.982	(0.0001)	0.083		
Time.*M. nivale*											
T1	0.307	(2.03)	-0.280	(0.525)	0.233	(1.71)	-0.264	(0.544)	0.344	0.341	0.329
T2	-0.589	(0.257)	-0.607	(0.247)	-0.074	(0.844)	-0.260	(0.550)			
T3	-1.361	(0.044)	-0.094	(0.806)	0.032	(1.08)	-0.161	(0.691)			

* - Only significant ($P \leq 0.05$) effects on pathogen and/or diseases are presented. SED[a] for comparing means between all factor levels, SED[b] for comparing means between growth stages within the same level of $Temp_{min}$. SED[c] for comparing means between $Temp_{min}$. [-] - Number of observations per factor level. [1] – Disease expressed as disease index (%). [2] – Pathogen DNA in soil expressed as pg g^{-1} of soil. [3] – pathogen DNA in *planta* expressed as pg ng^{-1} of total DNA. T1 – GS 21-31 (*n* = 102), T2 – GS 37-45 (*n* = 102), T3 – GS 65-75 (*n* -102).

Table 4.19 Effect of total rainfall (mm) over the autumn/winter period on pathogen DNA in soil and disease. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

Total rainfall (mm)*	**<199 [26]**		**200-399 [57]**		**400> [19]**		**SED[a]**	**SED[b]**	**SED[c]**
Disease[1]									
Time.Root rot									
T1	10.2		13.6		16.4		1.4	1.4	1.4
T2	11.2		8.4		9.6				
T3	3.4		3.1		3.9				
Soil[2]									
Time.*O. yallundae*									
T1	0.319	(2.08)	-1.013	(0.097)	-2.445	(0.004)	0.624	0.659	0.527
T2	-1.699	(0.020)	-1.152	(0.070)	-1.764	(0.017)			
T3	-0.112	(0.773)	-1.715	(0.019)	-1.62	(0.024)			

* - Only significant ($P \leq 0.05$) effects on pathogen and/or diseases are presented. SED[a] for comparing means between all factor levels. SED[b] for comparing means between growth stages within the same level of total rainfall. SED[c] for comparing means between total rainfall groups. [-] - Number of observations per factor level. [1] – Disease expressed as disease index (%). [2] – Pathogen DNA in soil expressed as pg g^{-1} of soil. T1 – GS 21-31, T2 – GS 37-45, T3 – GS 65-75.

Table 4.20 Effect of mean relative humidity (%) over the autumn/winter period on pathogen DNA in stem bases. Values are REML-predicted means from wheat crops sampled at T1 (GS 21-31), T2 (GS 37-45) and T3 (GS 65-75) in England during 2011/12 and 2012/13. DNA values are log_{10} transformed with back-transformed means in parenthesis (-).

Relative humidity (%)*	**<82% [22]**		**82.1-84.9 [41]**		**85> [39]**		**SED**
Stem bases[1]							
M. majus	-0.383	(0.414)	-0.364	(0.433)	-0.697	(0.201)	0.185

* - Only significant (P≤0.05) effects on pathogen and/or diseases are presented. [-] - Number of observations per factor level. [1] – Pathogen DNA in *planta* expressed as pg ng^{-1} of total DNA. T1 – GS 21-31 (*n* = 102), T2 – GS 37-45 (*n* = 102), T3 – GS 65-75 (*n* -102).

4.3.6 Effect of fungicides applied at GS 31-32 (T1) and GS39 (T2) on pathogen DNA and stem base diseases at GS 37-45 and GS 65-75

REML analysis could not be used to analysis the effect of fungicides applied at T1 and T2 on pathogen DNA in soil and planta and stem base diseases at GS 37-45 and GS 65-75. Instead fungicides were investiagted using an unbalanced ANOVA. T1 fungicides had a signfcant effect on BFR at GS 39 (Figure 4.1). The lowest BFR was found with T1 fungicide combinations of MSI + prothioconazole + strobilurin (24%) was the highest with SDHI + triazole + other (35%) combinations. The SED (min) showed that BFR index at GS 37-45 was lower after prothioconazole (26%) than Boscalid (31%) applied at at T1. There were no other significant effects of fungicides applied at T1 or T2 on pathogen DNA or stem base diseases.

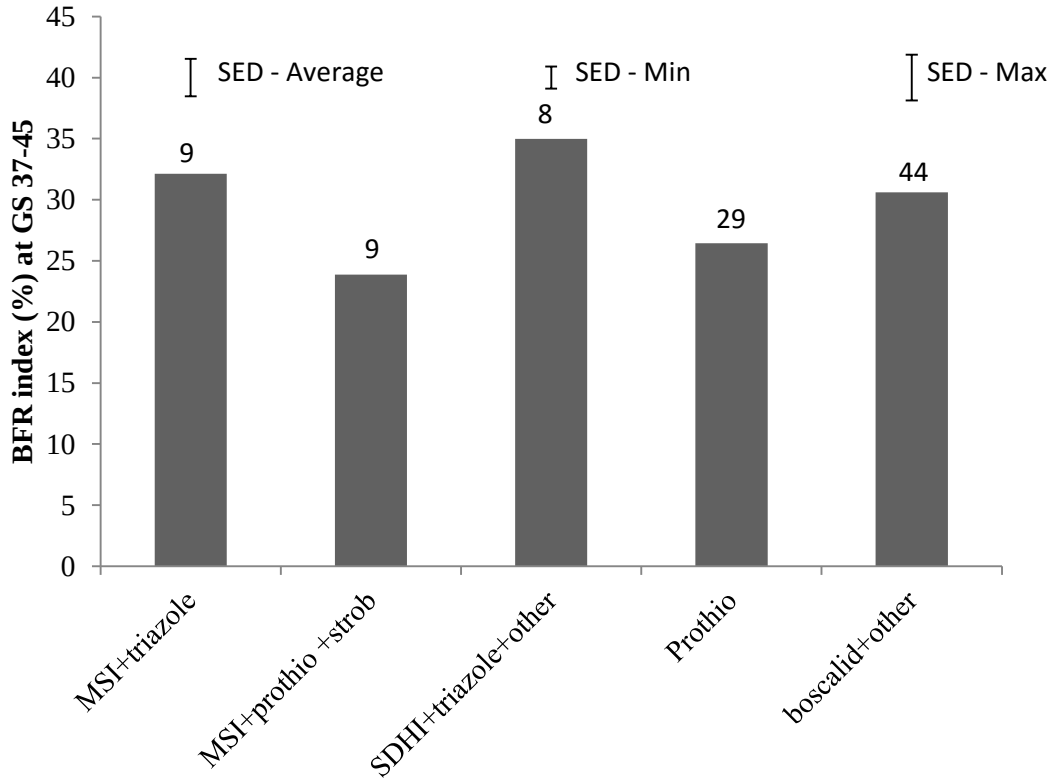


Figure 4.1 The effect of T1 fungicides on brown foot rot (BFR) at GS 37-45. MSI - multi-site inhibitors, Prothio – prothioconazole, Strob – strobilurin. Numbers above bars are number of observations. SED – Average for comparing all means. SED – Min for comparing means for the two highest number of observations, SED – Max for comparing the means of the two lowest number of observations.

4.4 DISCUSSION

Understanding how strongly agronomy practice and meteorological factors affect diseases and pathogens is important if growers are to implement effective management strategies to reduce root rot and SBD's and the development of associated SBD pathogens. This study also provides new information on agronomy practices that are influencing *R. solani* AG 2-1 in English wheat crops.

The main disease influenced by soil texture was sharp eyespot. Previous studies have shown sharp eyespot occurs over a wide range of soil textures but it is thought that the disease is favoured by sandy soils (Parry, 1990; Daamen and Stol, 1990). In this study sharp eyespot also occurred over a wide range of soil textures but showed no preference for sandy soils which had similar disease index as clay soils. However, sharp eyespot index and *Oculimacula* spp. DNA in soil and roots were lower in soils with high silt content suggesting a suppressive effect. Soil texture can affect diseases and pathogens due to differences in water holding capacity, nutrient status, root growth and growth of fungal species (Gill *et al.,* 2000; Ghorbani *et al.,* 2008). Therefore, this effect could be due to a number of indirect factors that occur in soils with high silt content.

Previous crop can also indicate which diseases are likely to be more prevalent due to the amount of inoculum that is carried over in soil and residues (Polley and Thomas, 1991; Colbach *et al.,* 1997a). In this study previous crop did not influence the severity of root rot or SBD's. However, quantifying species DNA in soil and *in planta* can reveal how the previous crop has affected pathogen population dynamics (Wale, 2004; Sparrow *et al.,* 2015). Sparrow *et al.* (2015) monitored changes in AG 2-1 DNA in soil of Australian potato fields over eight years. Soil samples were collected annually and AG 2-1 DNA increased when the previous crop was poppy and potato. In this study AG 2-1 DNA concentrations were highest following OSR and lowest after continuous wheat. *R. solani* AG 2-1 is a known pathogen of *Brassica* spp. (Verma, 1996; Babiker *et al.,* 2013) and is an important disease of canola in Canada causing considerable damping-off and root rot (Gugel *et al.,* 1987; Yitbarek *et al.,* 1987). The frequency of OSR in UK rotations has increased over the last 10-15 years. Historically OSR was grown one in five rotations with cereals but more recently OSR is grown in one in two or one in three years (AHDB, 2014). Shorter

rotations allow pathogens to thrive in soil if their preferred host occurs frequently. It is likely that increased frequency of OSR in the rotation is selecting for AG 2-1 in the soil. There is some evidence to support this view. A recent study utilising computed tomography (CT) to visualise the effect of AG 2-1 on the root architecture of wheat and OSR on days 2, 4 and 6 after sowing also extracted DNA from soil at the end of the experiment (day 6). Results showed a rapid increase in AG 2-1 DNA levels in soil when OSR was grown but a two-fold reduction for wheat (Sturrock *et al.,* 2015). This could have serious implications for OSR production in the UK as there is no known genetic resistance (Kataria and Verma, 1992).

The position of wheat in the rotation significantly influenced the development of diseases and pathogen DNA *in planta.* This was most notable for eyespot caused by *Oculimacula* spp. Eyespot severity was reduced and *O. acuformis* and *O. yallundae* DNA concentrations in roots and stem bases were lower in 1st wheats compared to 2nd and CW. A previous survey of 300-400 wheat crops in the UK found eyespot severity was generally less severe in 1st and 2nd wheats compared to CW (Polley and Thomas, 1991). However, Hardwick *et al.* (2001) sampled 360-450 UK wheat crops and found eyespot severity was not affected by the number of preceding wheats crops only by a break from wheat. Therefore, this study was in agreement with Hardwick *et al.* (2001) showing that a break from wheat reduces eyespot severity. Even so eyespot severity at the end of the season was lower in 1st wheats due to a significant decline between GS 37-45 and GS 65-75. The pattern of development shown in 1st wheats is the same as the overall trend in 2011/12 while in the following season eyespot failed to develop suggesting a possible interaction with an agronomy practice or the environment in 2011/12. Interactions were only investigated with time (growth stage) in this study to investigate differences in development of diseases or pathogens. In addition, interactions between agronomy factors could not be investigated due to limited degrees of freedom for the majority of factor combinations. However, DNA of *O. yallundae* in stem bases significantly increased at each growth stage in 1st wheats suggesting a lack of relationship between eyespot severity and *O. yallundae* DNA. This was also shown in 2nd wheats at GS 65-75 where the highest *O. yallundae* DNA concentrations occurred in stem bases but eyespot

severity had declined slightly compared to GS 37-45 levels. Therefore, the effect of position of wheat in the rotation had contrasting effects on disease severity and pathogen DNA.

Interestingly cultivation in this study had no significant effect on disease development or pathogen DNA in soil or *in planta*. There have been numerous studies that have shown cultivation is one of the main agronomy factors to influence diseases and pathogens (MacNish, 1985; Pumphrey *et al.,* 1987; Prew *et al.,* 1995; Smiley *et al.,* 1996; Bateman *et al.,* 1998; Jenkyn *et al.,* 2001; Jenkyn *et al.,* 2010). In experimental plots ploughing that inverts soil burying infected crop residues reduced the incidence of BFR (Bateman *et al.,* 1998). Ploughing is also thought to fracture the hyphal network of *Rhizoctonia* spp. reducing the amount of inoculum available to cause infections (MacNish, 1985; Paultiz *et al.,* 2002). In contrast, minimum tillage that leaves crop residues on the surface has been shown to reduce the amount of eyespot and sharp eyespot infections (Prew *et al.,* 1995; Jenkyn *et al.,* 2001). Clearly cultivation is a major factor influencing diseases and pathogens. It is not clear why there was no effect of cultivation in this study and this could be due to a number of factors including; not enough fields to detect differences, potential interaction with other variables such as previous crop, seed treatments or fungicides or the relatively short time period (two seasons) this study was conducted. However, there could be interactions with other factors such as previous crop. Colbach *et al.* (1997b) showed the effect of cultivation on sharp eyespot incidence depended on the previous crop. When a host was grown previously ploughing reduced sharp eyespot but if the previous crop was a non-host itself preceded by a host then ploughing increased sharp eyespot by bringing infected material back to the surface. Similarly Jenkyn *et al.* (2010) showed that minimum tillage *per se* is not responsible for reducing eyespot. This was demonstrated by applying residues to ploughed and tined cultivated plots and found eyespot was reduced regardless of cultivation method; suggesting the mechanism was the build-up of antagonistic microbes in crop residues.

Sowing date is considered one of the main agronomy practices that can influence infections and disease development (Clarkson and Cook, 1983; Polley and Thomas, 1991; Colbach *et al.,* 1996; 1997a; 1998; Burnett *et al.,* 2012). Previous studies have shown eyespot, BFR and sharp eyespot incidence and/or severity is increased in earlier sown crops and decreased in later sown crops (Colbach *et al.,* 1996; 1997a; 1998; Burnett *et al.,* 2012). Greater disease in early sown crops is usually explained by the longer periods of favourable conditions for pathogens to infect crops before the onset of winter (Colbach *et al.,* 1996; 1997a; 1998). In this study crops sown early (1^{st}-14^{th}) September had higher root rot, eyespot and BFR which all showed a decreasing trend until the last sowing dates in November. Therefore, this in agreement with previous studies that early sown crops are at greater risk of infections than late sown crops (Polley and Thomas, 1991; Colbach *et al.,* 1996; 1997a; Burnett *et al.,* 2012). Previous studies have also shown early sowing is one of the main factors increasing sharp eyespot infections (Clarkson and Cook, 1983; Polley and Thomas, 1991; Colbach *et al.,* 1997a). In this study sowing date was not identified as a significant factor influencing sharp eyespot severity. DNA concentrations of *M. nivale* (soil), *O. acuformis* (roots) and *O. yallundae* (roots and stem bases) were all higher in early September sown crops than November. This provides evidence that earlier sown crops are colonised to a greater extent than later sown crops. However, growers will be reluctant to delay sowing as soil conditions can become unconducive to field operations especially into late October and November. To reduce infections in early sown crops growers can use resistant varieties and seed treatments. This study showed that varieties with an eyespot resistance (ER) rating of 8 had the lowest *O. yallundae* DNA concentrations in stem bases. In chapter 3 *O. yallundae* was identified as the predominant *Oculimacula* spp. in English wheats crops thus to reduce infections control of this species is vital. Varieties with an ER rating of 8 in this study were Skyfall and Grafton that are believed to carry the *Pch*1 gene conferring eyespot resistance (Burnett *et al.,* 2012). That said this was only shown with *O. yallundae* and not for *O. acuformis* or with visual eyespot index.

Prothioconazole was one of the most frequently occurring active ingredients used in seed treatments in this study. *Oculimacula* spp. are known to be sensitive to

prothioconazole (Burnett *et al.,* 2012) but in this study had no significant influence on eyespot or the *Oculimacula* spp. DNA. Fludioxonil and prothioconazole are actives ingredients with good efficacy towards *Microdochium* spp. (Dutzmann and Suty-Heinze, 2004; Glynn *et al.,* 2007; Glynn *et al.,* 2008). However, in this study seed treatments that contained these actives were not shown to influence the *Microdochium* spp. This could be due to samples being collected too late (GS 21-31) to detect any seed treatment effect. Therefore, an earlier sampling may have detected differences.

The environmental conditions over the autumn/winter period influence the ability of pathogen to infect crops and therefore disease development in the spring and summer. Temperature over the autumn/winter period in this study had contrasting effects on the development of diseases and pathogens. At GS 21-31 root rot, eyespot and sharp eyespot severity were highest in areas that had the warmest autumn/winter period. This will be due to warmer conditions allowing greater activity of pathogens leading to enhanced disease severity. Sharp eyespot severity increases in warmer autumn/winter periods as these conditions allow greater infection and colonisation of stem by *R. cerealis* (Lemańczyk, 2012). In addition, eyespot incidence in field experiments has been positively correlated with thermal time (Wan *et al.,* 2005). However, the development of eyespot and sharp eyespot severity generally declined between GS 37-45 and GS 65-75 in the areas that had a warmer autumn/winter. This could be due to greater loss of outer leaf sheaths (Fitt *et al.,* 1998) in the spring and summer due to more advance growth in warmer areas. Environmental factors did influence the *Oculimacula* spp. DNA suggesting areas that had the warmest autumn/winter period (>10°C) restricted the development of *O. yallundae* (soil) and *O. acuformis* (stem bases) compared to cooler areas (<8.99°C). Warmer winters are generally thought to increase pathogen activity. However, temperatures of >10°C have been shown to stimulate the growth of antagonistic fungi on straw naturally infected with the *Oculimacula* spp. (Higgins and Fitt, 1984). This could explain the lower *Oculimacula* spp. at warmer autumn/winter temperatures. In contrast the *Microdochium* spp. are known to be remarkably active at low temperatures, causing disease even in freezing conditions (Millar and Colhoun, 1969; Pettitt *et al.,* 1996; Haigh and Hare, 2012). In soil, *M. nivale* DNA concentrations were

favoured by colder and *M. majus* by relatively warmer conditions. The base temperature for the growth on PDA plates of *M. nivale* and *M. majus* was calculated at −6.3◦C and −2.2◦C, respectively (Haigh and Hare, 2012). This shows the greater metabolic activity of *M. nivale* at low temperatures compared to *M. majus*. DNA levels of *M. nivale* in stem bases were also higher than *M. majus* at GS 21-31. Imathiu *et al.* (2010) showed in an controlled environment study that *M. nivale* caused seedling blight at 5°C and 15°C compared to *M. majus* that failed to cause disease at 5°C and was only weakly pathogenic at 15°C. This study under field conditions showed greater activity of *M. nivale* at lower temperatures compared to its related species *M. majus.* Infection due to *Microdochium* spp. over the autumn/winter period can impact on crop development. In a semi-controlled environment study, *M. nivale* and *M. majus* infections at the seedling stage under cold conditions (3°C) later resulted in reduced plant vigour (e.g. stem length, dry weight and grain yield) (Haigh *et al.,* 2009). Therefore, controlling these species at the seedling stage is important to prevent yield losses due to seedling blight and to reduce seed infections as these species can be splashed dispersed up to the ear (Haigh *et al.,* 2009). The information of how environmental conditions over the autumn/winter period influence disease and pathogens could be used to inform foliar fungicides at T1 (GS 31-32) and T2 (GS 39).

However, the only effect of foliar fungicides applied at T1 and T2 in this study was on BFR. Fungicides applied at T1 that contained prothioconazole had lower BFR index at GS 37-45 than other treatment combinations. Prothioconazole applied as a foliar treatment has shown to reduce Fusarium head blight which is caused by a range of *Fusarium* spp. including *M. nivale* and *M. majus* (Edwards and Godley, 2010). Fungicides in this study were grouped on their activity to eyespot with treatments based around prothioconazole, boscalid and epoxiconazole. Prothioconazole and boscalid are the two most commonly applied fungicides in commercial wheat crops to control eyespot (Burnett *et al.,* 2012). Epoxiconazole also gives good control of eyespot mainly due to its greater activity on *O. yallundae* than *O. acuformis* (Burnett *et al.,* 2012). However, this study found no effect of foliar fungicides applied at T1 and T2 on eyespot index or *Oculimacula* spp. DNA *in planta* at GS 37-45 and GS 65-75

respectively. There was also no effect of fungicides on sharp eyespot severity at GS 37-45 and GS 65-75. Similarly, field experiments in Poland applied a range of active ingredients (flusilazole, fuberidazole, imazalil, picoxystrobin, propiconazole and triadimenol) at standard agronomic timings and found that none decreased sharp eyespot infections (Lemańczyk, 2012). However, chemical control is often variable (Parry, 1990; Ray *et al.*, 2004) and it is unlikely any of the fungicide applications in this study were specifically applied to control sharp eyespot as it is generally considered of low importance. This study found no effect on foliar fungicides on pathogen DNA in stem bases.

4.5 CONCLUSIONS

The results within this chapter enable the following conclusions to made:

i. The highest concentrations of AG 2-1 DNA occurred when the previous crop grown was OSR. This suggests that OSR is selecting of AG 2-1 in the soil.

ii. *Rhizoctonia cerealis* DNA was not effected by any agronomy practice or meteorological variables in this study suggesting other factors not investigated in this study influenced this pathogen.

iii. Sowing crops early (September) resulted in greater eyespot and BFR severity and increases in *Oculimacula* spp. and *M. nivale* DNA in soil and *in planta* than those found in later sown crops (November).

iv. Eyespot severity and *Oculimacula* spp. DNA was higher in 2nd and CW than 1st wheats. This was most notable in 2nd wheats were high levels of *O. yallundae* DNA occurred although this did not result in greater eyespot severity.

v. Warmer autumn/winter period reduced the amount of *O. yallundae* (soil) and *O. acuformis* (stem bases) DNA detected which could be due to greater build-up of antagonistic microbes.

vi. *Microdochium nivale* was favoured by colder conditions over the autumn/winter period. This was shown with higher DNA concentrations in stem bases at GS 21-31 compared to the other species in this study.

vii. Crops that were treated at T1 with a fungicide mixture that contained prothioconazole had lower BFR severity at GS 37-45. There was no effect of fungicides on the other SBDs or associated pathogens.

Chapter 5

Elucidating crop losses and control of *Rhizoctonia* spp. in field grown winter wheat

5.2 INTRODUCTION

Rhizoctonia solani AG 2-1 (63%), *R. cerealis* (54%) and AG 5 (5%) were previously identified as the three most commonly occurring *Rhizoctonia* spp. detected in the soil of English winter wheat crops (Chapter 3). Field experiments conducted during 2012/13 and 2013/14 were designed to investigate the effects of disease caused by AG 2-1, *R. cerealis* and AG 5 on crop growth and yield of winter wheat and to determine the effectiveness of a fungicide seed treatment from the class of succinate dehydrogenase inhibitors (sedaxane).

Rhizoctonia cerealis is the causal agent of sharp eyespot which is a commonly occurring stem base disease in UK wheat crops (Polley and Thomas, 1991; Polley and Turner, 1995; Hardwick *et al.,* 2001). Severe disease on stems interrupts the transport of water and nutrients to the developing ears leading to the occurrence of whiteheads, stem lodging and ultimately loss of yield. Yield loss due to sharp eyespot is only associated with moderate or severe lesions caused by the pathogen. Clarkson and Cook (1983) on average found moderate and severe infections reduced yield by 5 and 26% respectively at 18 sites in England and Wales from 1976 to 1981. In New Zealand yield losses were negligible when the number of tillers infected was <10% but maximum individual yield loss in a crop was estimated at 18% (Cromey *et al.,* 2002). More recently Lemańczyk and Kwaśna (2013) estimated maximum yield losses in Poland were 8-10% when >50% of stems were infected with 25% of these infections rated as moderate or severe. However, *R. cerealis* is also able to cause pre- and post-emergence damping-off but this had been investigated less thoroughly (Parry, 1990). In addition, there is very low awareness by growers/agronomists that *R. cerealis* can cause establishment losses. Recently it has been proposed that since 1980 the incidence and severity of sharp eyespot has increased globally resulting in yield and quality losses (Hamada *et al.,* 2011; Lemańczyk and Kwaśna 2013). However, the effect of *R. cerealis* on the yield of wheat in the UK has only been investigated by collecting infected samples from grower's fields at harvest (Clarkson and Cook, 1983). Therefore, there is a lack of knowledge about the effect of *R. cerealis* on the growth of winter wheat from emergence to harvest yield.

There are limited and contrasting reports on the pathogenicity of isolates of AG 2-1 and AG 5 to wheat. Roberts and Sivasithamparam, (2006) in Western Australia isolated 27 AG 2-1 isolates from wheat roots affected by bare patch disease. Two of these isolates were screened in pathogenicity experiments and were shown to cause significant root rot. In Turkey Demirci, (1998) isolated five AG 2-1 isolates from the crown and subcrown internode tissue of wheat and barley. Four of these isolates were found to be moderately virulent causing superficial discolouration or small lesions on 30 day old wheat seedlings. However, Sturrock *et al.* (2015) used computed tomography to investigate the effect of one AG 2-1 isolate, obtained from arable soil in the UK, on damping-off of wheat. This study scanned plants at 2, 3 and 6 days following inoculation (dfi) and found root architecture traits such as the number of primary roots and root volume were significantly reduced. However, visual disease symptoms were only observed at 6 dfi and classified as slight (small lesions on the primary roots). Other studies have reported that isolates of AG 2-1 are very weak to non-pathogenic towards wheat seedlings. Khangura *et al.* (1999) investigated the pathogenicity of one AG 2-1 isolate taken from OSR roots in Western Australia and found it caused no visible symptoms on 21 day old wheat seedlings. In an earlier study also in Western Australia three AG 2-1 isolates taken from wheat/lupin roots were found to be weak or non-pathogenic to 20 day old wheat seedlings (Sweetingham *et al.,* 1986). This was also found in South Africa where three isolates of AG 2-1 were non-pathogenic on 14 day old wheats seedlings (Tewoldmedhin *et al.,* 2006).

In Texas Rush *et al.* (1994) investigated three AG 5 isolates taken from sugar beet and wheat roots and found they had no effect of emergence of seven day old wheat seedlings but did cause significant disease on the roots. In the UK AG 5 was isolated from brown lesions on wheat stems taken from a field in Shropshire, England. In a controlled environment study this isolate caused brown lesions on wheat stems after three weeks growth. However, in the Pacific Northwest of America Ogoshi *et al.* (1990) found two AG 5 isolates taken from wheat roots caused no disease or plant stunting at 10°C or 20°C on 21—35 day old wheat seedlings.

These studies show the variation in pathogenicity that exists between isolates of AG 2-1 and AG 5 to wheat. This has also been shown in other crops. For example, Woodhall *et al.* (2007) observed large variation in disease on roots and stems of potatoes from seven AG 2-1 isolates. In addition, the majority of studies on the effects of AG 2-1 and AG 5 on wheat have been conducted on seedlings in controlled environments. Therefore, the effect of these pathogens on the growth of wheat crops under field conditions has not previously been investigated.

Control of *Rhizoctonia* spp. at the establishment phase of crop development is critical as early infections can lead to seedling death resulting in substantial thinning out of stands. Therefore, seed treatments are the most effective control options during the seedling period when plants are most susceptible to the disease. Ideally a seed treatment should be systemic, highly persistent and able to control all species and AG's (Kataria and Gisi, 1999; Hamada *et al.,* 2011b). Sedaxane is a new broad spectrum fungicide developed by Syngenta Crop Protection (Zeun *et al.*, 2013). The mode of action of sedaxane is the inhibition of the succinate dehydrogenase enzyme in complex II of the mitochondrial respiration chain (Cecchini, 2003; Oyedotun and Lemire, 2004). Glasshouse studies have shown sedaxane is highly active against *R. solani* on several crop species (Zeun *et al.*, 2013). In addition, field plots artificially inoculated with *R. cerealis* showed a yield advantage from treatments containing sedaxane (Zeun *et al.,* 2013).

In this chapter a range of measurements (visual, molecular and physiological) were conducted from emergence through to harvest yield to establish the effects of AG 2-1, AG 5 and *R. cerealis* and fungicide seed treatments on biomass and yield of winter wheat.

The aim of these field experiments were to determine the effects of AG 2-1, AG 5 and *R. cerealis* on the biomass and yield of winter wheat and to evaluate the effectiveness of two fungicide seed treatments using visual, real-time PCR analysis and physiological assessments. Relationships were also investigated between rhizoctonia disease severity, fungal biomass (DNA), physiological parameters and yield.

The individual objectives of the 2012/13 and 2013/14 field experiments were to:

i) Quantify emergence losses of winter wheat to AG 2-1, AG 5 and *R. cerealis* and to evaluate the response of seed treatments (fludioxonil or sedaxane + fludioxonil) to reduce any establishment losses from the species.

ii) Investigate diseases caused by AG 2-1, AG 5 and *R. cerealis* on the roots and stems of winter wheat.

iii) Assess the effect of AG 2-1, AG 5 and *R. cerealis* and seed treatments on the physiology, biomass and yield of winter wheat.

iv) Determine the effectiveness and response from seed treatments to control disease caused by AG 2-1, AG 5 and *R. cerealis*.

The specific null hypotheses tested within this chapter are:

i) *Rhizoctonia* spp. and seed treatments will have no effect on emergence of winter wheat.

ii) Disease on the roots and stems of wheat plants will not be related to *Rhizoctonia* spp. and fungicide seed treatments.

iii) There will be no effect on DNA concentrations of Rhizoctonia spp. in roots and stems of winter wheat by seed treatments.

iv) The physiology of winter wheat will not be affected by *Rhizoctonia* spp. and seed treatment.

v) *Rhizoctonia* spp. and seed treatments will have no effect on the yield components of wheat.

vi) There will be no relationships between diseases, pathogens or physiological parameters and yield.

5.2 Materials and methods

5.2.1 Experimental design

Field experiments using a randomised block design were conducted in 2012/13 and 2013/14 (Appendix 5). Two experimental fields were chosen, S26 (latitude 52.835258; longitude 1.251422) in 2012/13 and S2 (latitude 52.829632; longitude -1.244127) in 2013/14. The sites have a maritime temperate climate and the soil texture is classified as sandy loam. Soil was mixed with artificial inoculum produced as described in 5.2.2 from *R. solani* AG 2-1 (isolate 1917), AG 5 (isolate 1906) and *R. cerealis* (Rc isolate 1480). Inoculum, grown on millet seed, was sown at a rate of 30 g m^{-2} of millet seed per plot sown together with the wheat seed (c.v Santiago). Appendix 10 shows details of the origin of isolates. Wheat seed was treated with sedaxane (10 g a.i 100 kg^{-1}) + fludioxonil (5 g a.i 100 kg^{-1}), fludioxonil (5 g a.i 100 kg^{-1}) (Beret gold®) or untreated. The experiment was designed with twelve treatment combinations of pathogen inoculation (x4) and seed treatment (x3) (Table 5.1). In 2012/13 and in 2013/14 there were four and three replications of each treatment combination, respectively. The difference in replications was because in 2013/14 the experiment was re-established due to an earlier mechanical fault with drill. Prior to the start of each experiment soil samples from the experimental area were collected and DNA extracted (refer to section 2.2.1 for soil DNA extraction method) then AG2-1, AG 5 and *R. cerealis* targeted using real-time PCR assays (section 2.3.1).

Table 5.1 Inoculation and seed treatment combinations for 2012/13 and 2013/14 field experiments

Inoculation	**Seed treatment**
1. Not inoculated (Control)	1. Untreated
2. AG 2-1	2. Sedaxane 10 g a.i 100 kg^{-1} + fludioxonil 5 g a.i 100 kg^{-1}
3. AG 5	3. Fludioxonil 5 g a.i 100 kg^{-1}
4. *R. cerealis*	

5.2.2 Inoculum production

Inoculum was grown on millet seed following the method described by Zeun *et al*. (2013). All isolates were stored on PDA slopes at 15°C before being sub cultured onto PDA plates. After 6-7 days growth three agar plugs of each isolate were removed and placed in 5% sterile Sabouraud Maltose Broth (SMB). Flasks were then sealed and incubated in the dark for 14 days at 22°C. The mycelium was washed with sterile water to remove excess broth and then transferred to a sterile 500 ml glass beaker and mixed aseptically in 100 ml of sterile deionised water with an Ultra Turrax® high performance disperser. Millet seed (2 kg per isolate) and 500 ml of deionised water were added to autoclave bags and sterilised at 121°C for 1 h on two consecutive days. 200 ml of mycelium suspension were then added to each bag of sterile millet seed and thoroughly mixed. Inoculated millet seed was spread in a thin layer within the bag and loosely sealed with a cotton wool bung and then incubated at 22°C in the dark for 14 days. After 14 days the seed was spread out on plastic trays and covered with plastic and incubated for a further two days at 20°C. The plastic covers were then removed and the seed left to air dry for four days.

5.2.3 Agronomy

In both experiments winter wheat was sown at a rate of 320 seeds m^2. Plots were drilled with a Wintersteiger plot drill which allowed the inoculated millet seed to be sown alongside the wheat seed (Figure 5.1). Plot size was 6 x 1.6 m in 2012/13 field experiment and 1 x 1 m in 2013/14. The experiments were sown on the 4th October and 24th October in 2012 and 2013, respectively. Crop

protection measures followed standard agronomic practices except for the fungicide programme which was designed to give robust protection against foliar and stem base diseases utilising active substances that were not active towards AG's of *R. solani* and *R. cerealis* (appendix 6).



Figure 5.1 Wintersteiger plot drill used in the 2012/13 field experiment

5.2.4 Plant sampling and crop assessments

Crop growth stages were assessed according to the decimal code of Zadoks *et al.* (1974). The crop was adjudged to have reached a specific growth stage when ≥50% of the plants had been assigned to it. Individual crop growth and physiological measurements, and disease assessments were performed at GS 15, GS 31, GS 39, GS 75 and GS 85.

At GS 15 and GS 31 fifteen plants were randomly selected per plot. Later at GS 39 and GS 75 all plants were removed from within an area of 0.25^2 m per plot randomly selected using a quadrat and subject to detailed biomass assessment (section 5.2.11). At each sampled growth stage plants were removed retaining all above ground biomass and as much of the top 15 cm of the root system as feasible. Samples were placed into labelled clean plastic bags in the field and then transferred to cold storage room (4°C) until disease assessments and crop measurements were performed (not more than 48 h). Prior to assessments all roots and stems were thoroughly washed to remove any debris.

5.2.5 Seedling emergence and assessment of plant populations

Seedling emergence counts were conducted four weeks after sowing. Plants were counted within a 0.25 m^2 quadrat (0.5m x 0.5m) in the centre of plots away from the edge. Three counts per plot were taken to obtain an average. This was then converted into a percentage of the 320 seeds m^2 sown. Plant populations were again assessed at GS 39. At this time all plants were removed from within a 0.25 m^2 quadrat and counted in the laboratory.

5.2.6 Visual disease assessments

Visual disease assessments on the roots and stems were conducted at GS 15, GS 31, GS 39 and GS 75 on 15 plants per plot. At GS 15 and GS 31 all tillers were assessed for disease and at GS 39 and GS 75 only the main stem was assessed for disease. Plants were assessed for root rot, sharp eyespot, eyespot and brown foot root on the stem bases. Disease assessment keys for each disease are described in appendix 2. At GS 15 it was not possible to distinguish between stem base diseases therefore stems were assessed for generally stem browning based on the key given in appendix 2.

5.2.7 Soil sampling and pathogen DNA extractions

DNA extraction from soil was only conducted on samples collected at GS 15. Five soil cores per plot were removed then homogenised and stored at -80°C until DNA extractions could be performed. All soil DNA extractions were performed on 50 g samples (refer to section 2.2.1 for soil DNA extraction method).

5.2.8 Pathogen DNA extraction from plant material

Due to the small sample size of stems collected at GS 15 DNA was extracted using BIOREBA® extraction bags. Stems (5 cm stem basal region) from 15 plants were freeze dried and stored until DNA extraction could be performed. Samples were weighed and then placed in a BIOREBA® extraction bag. Bags were then placed in liquid nitrogen (N_2) for 10-15 seconds removed and the stems broken-up with the use of a rubber mallet. CTAB buffer (6 ml) (as for soil

CTAB) was added to the bag, these were then macerated using a BIOREBA Homex 6 flatbed grinder. Plant material was allowed to settle (2-3 minutes) and the resulting supernatant was transferred to a 2ml tube and centrifuged at 2000g for 2 minutes. Then 700 µl of clear lysate was transferred to a 2 ml tube containing 200 µl chloroform and vortexed until the mixture turned turbid. These were then centrifuged at 13000g for 5 minutes. Then 500 µl of the resulting supernatant was added to the first well of a Kingfisher tube set with 500 µl isopropanol and 50 µl magnetic beads. The second well contained 1 ml buffer B (Wizard Food kit), third and fourth well 1 ml 70% ethanol and the final well 200 µl TE buffer. Extractions were performed using the 'Genomic DNA' program of the Kingfisher ML magnetic particle processor (Thermo Electron Corporation). Extracted DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA).

At GS 31 and GS 75, DNA was extracted from the roots and stems of 15 plants per plot. DNA was extracted from 10 cm stem basal region at GS 31 (all tillers) and 15 cm at GS 75 (main stems only). The DNA extracted method is presented in section 2.2.2.

5.2.9 Pathogen quantification using real-time PCR

Real-time PCR assays were only performed for AG 2-1, AG 5 and *R. cerealis* from DNA extracted from plants grown in plots inoculated with the aforementioned pathogens. No real-time PCR assays were performed from plants grown in control plots (non-inoculated). Primers and probes and PCR cycle conditions are presented in Appendix 1.

5.2.10 Green area index, dry weight and ear partitioning index

At GS 39 and GS 75 all plants were removed from a 0.25 m^2 quadrat for detailed laboratory based assessment of above ground plant biomass. Roots were removed from each plant and the sample weighed for fresh weight (FW). A subsample of 10% (by fresh weight) of plants was then selected and partitioned into leaf lamina (L) (flag leaf, 2^{nd} leaf, remaining laminar), true stems (S) and

ears (E) (GS 75 only). The green area (GA) for each component part was measured (cm^2) using a LI-3100C Area Meter (LI-COR Lincoln, Nebraska US). Then fresh weight of each component part was recorded before samples were dried in a ventilated/forced draft oven at 80°C until a constant weight was achieved, generally 72 hrs. Dry weight (DW) was then recorded on each component part. This data allowed calculations of green area index (GAI), above ground dry weight (AGDW) and ear partitioning index (EPI) as per equations 1 to 5:

The green area index (GAI) defined as the green canopy area per unit ground area and is a precise way of estimating the light-capturing capacity of a canopy (Pask *et al.,* 2012). The green area index (GAI) was calculated as per equation 1 and 2:

GAI (GS39) = ((FW/10% of FW)*(GAL+GAS))/0.25/10000 (Equation 1)

GAI (GS75) = ((FW/10% of FW)*(GAL+GAS+GAE))/0.25/10000 (Equation 2)

Above ground dry weight (AGDW) was then calculated as per equation 3 and 4:

AGDW (GS 39) = ((FW/10% of FW)*(DWL+DWS))/0.25 (Equation 3)

AGDW (GS 75) = ((FW/10% of FW)*(DWL+DWS+DWE))/0.25 (Equation 4)

Ear partitioning index (EPI) defined as the fraction of above-ground DW at GS75 in the ear was calculated as per equation 5:

EPI (GS 75) = DWE/(DWL+DWS+DWE) (Equation 5)

5.2.11 Spectral reflectance assessments

A field spectrometer (CROPSCAN, *Inc.* Rochester USA) was used at GS 39, GS75 and GS85. Spectrometers are used to measure canopy reflectance, and have a typical spectral range of 350–1100 nm, these assessments can be used to provide information on the physiological status of the crop canopy (Pask *et al.,* 2012). Measurements were taken on sunny days with negligible wind and the plant leaves dry. The timing of measurements was close to solar noon typically between 11am and 1pm. The spectrometer allowed a range of spectral reflectance indices (SRI) to be calculated related to physiological processes (Table 5.2). Equations for indexes are presented in Appendix 7.

Table 5.2 Spectral reflectance indices (SRI) for wheat canopy analysis at GS39, GS75 and GS85 for 2012/13 and 2013/14 field experiments

Index	Name	Physiological process
NDVA	Normalized difference vegetation index	Green area, photosynthetic capacity, N status
R-NDVI	Red normalized difference vegetation index	Green area, photosynthetic capacity, N status
G-NDVI	Green normalized difference vegetation index	Green area, photosynthetic capacity, N status
SRa	Simple ratio	Green biomass
RARSa	Ratio analysis of reflectance spectra chlorophyll a	Chlorophyll b content
RARSc	Ratio analysis of reflectance spectra carotenoid	Carotenoid content
PRI	Photochemical reflectance index	Dissipation of excess radiation
WI	Water index	Plant water status
NWI-1	Normalized water index 1	Plant water status
NWI-2	Normalized water index 2	Plant water status
NWI-3	Normalized water index 3	Plant water status
NWI-4	Normalized water index 4	Plant water status

(Adapted from: Pask *et al.,* 2012)

5.2.12 Yield components

Plants height (cm^2), green area (GA) (cm^2) and number of roots were recorded at GS 15. Height was taken from the stem base to the top of the longest leaf. Green area was recorded using a LI-3100C Area Meter. At maturity (GS 93) plots were harvested with a Sampo plot combine equipped with a grain weighing system to establish total yield per plot before converting to tonnes per hectare. Grain samples were used to quantify thousand grain weight (TGW) and specific weight (SW) using a Sinar (model - AP6060-001AG) moisture analyser.

5.2.13 Statistical analysis

All data was analysed using Genstat® Version 17.1 for Windows (VSN International Ltd, UK). All DNA data was $\log_{10}$ transformed and disease index, where required, was angular transformed to normalise residuals. Where appropriate the back-transformed means of transformed data are presented in parentheses. Data was analysed using ANOVA to investigate AG x treatment x season interactions. To assess whether sharp eyespot index would be confounded by eyespot and BFR index, data on these diseases were included as covariates in the analysis. Physiological data was analysed with repeated measures ANOVA. Multiple linear regression (MLR) was used to analyse variation in disease, DNA concentration and yield. A probability value of 0.05 or less ($P \leq 0.05$) was taken to be significant, although values between 0.05 and 0.10 may receive comments in the text.

5.3 RESULTS

5.3.1 Emergence and plant populations

Four weeks after sowing seedling emergence was counted and presented as a percentage of the number of seeds planted (320 seed m^2). The three way interaction with main factors (season x inoculation x treatment) for emergence in the two experiments was significant ($P<0.001$), suggesting inconsistent effects across seasons (Table 5.3). In 2012/13 there were no significant differences in emergence from untreated seed in the inoculated and non-inoculated plots (range 50.6-54.2%) (Table 5.3). However, in 2013/14 emergence from untreated seed in the non-inoculated plots was high (81.3%) and there was no effect of AG 2-1 or AG 5 but *R. cerealis* significantly reduced emergence by 35% compared to the non-inoculated control. Seed treatments consistently resulted in increased emergence in 2012/13 in all plots but in 2013/14 there was no response to seed treatment in the non-inoculated plots. Sedaxane + fludioxonil was most active against *R. cerealis* significantly increasing emergence by 54% compared to the untreated. Emergence from fludioxonil in AG 5 inoculated plots was also increased by 4% compared to the untreated.

Final plant population (m^2) at GS 39 was higher in sedaxane + fludioxonil (207.4 m^2) and fludioxonil (196.3 m^2) treated plots compared to the untreated (170.7 m^2) ($P=0.001$) (Table 5.4).

Table 5.3 Emergence (%) of wheat seedlings 4 weeks after sowing. Seed was treated with sedaxane and/or fludioxonil or untreated and sown into field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*

Season	Treatment	Control	AG 2-1	AG 5	Rc
	UT	54.2	50.6	51.9	53.0
2012/13	F	81.6	73.3	80.2	75.0
	S + F	82.8	82.0	74.2	75.6
	UT	81.3	87.1	77.1	52.9
2013/14	F	83.8	79.6	80.0	57.5
	S + F	80.0	62.5	67.1	81.3

	P	df	SED[a]	SED[b]
Season	0.003	1	0.9	
Inoculation	<.001	3	2.3	
Treatment	<.001	2	2.0	
Inoculation*Treatment	0.014	6	4.0	
Inoculation*Season	0.034	3	3.0	3.3
Treatment*Season	<.001	2	2.5	2.9
Inoculation*Treatment*Season	<.001	6	5.6	5.8

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.4 Plant population (m^2) at GS 39 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*

Season	Treatment	Control	AG 2-1	AG 5	Rc
	UT	153	174	163	129
2012/13	F	201	174	184	184
	S + F	220	202	192	197
	UT	197	208	199	164
2013/14	F	232	211	239	160
	S + F	212	211	205	227

	P	df	SED[a]	SED[b]
Season	0.216	1	17.1	
Inoculation	0.146	3	11.2	
Treatment	0.001	2	9.73	
Inoculation*Treatment	0.392	6	19.5	
Inoculation*Season	0.828	3	22.1	16.1
Treatment*Season	0.416	2	20.6	13.9
Inoculation*Treatment*Season	0.6	6	31.7	27.8

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

5.3.2 Root rot disease and effect of seed treatments

Seed treatments consistently reduced root rot in both experiments at GS 15 (Table 5.5). Fludioxonil reduced root rot by 29.1% compared to the untreated. There was an additional effect of sedaxane + fludioxonil which reduced root rot by 35.1% compared to the untreated. However, the difference between seed treatments was not significant at $P \leq 0.05$.

At GS 31 there was a treatment x season interaction (P=0.046) (Table 5.6). This showed control of root rot was not consistent between experiments. In the 2012/13 experiment root rot was significantly higher in fludioxonil (20%) treatments compared to the untreated (13.6%).

Root rot was higher at GS 39 in 2013/14 (6.9%) compared to 2012/13 (3.7%) (P=0.039) experiments.

However, at GS 75 an AG x treatment interaction (P=0.038) was detected (Table 5.8). The interaction was due to the higher root rot in AG 5 inoculated plots from fludioxonil (11%) treatments comparted to the untreated (7.3%).

Table 5.5 Root rot disease index (%) at GS 15 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis)

Season	**Treatment**	**Control**		**AG 2-1**		**AG 5**		**Rc**	
	UT	13.9	(6.3)	15.3	(7.5)	9.0	(3.3)	10.1	(4.6)
2012/13	F	11.7	(5)	8.7	(5)	7.9	(2.5)	4.5	(1.3)
	S+F	6.3	(1.7)	9.6	(2.9)	6.9	(2.1)	6.3	(1.7)
	UT	6.0	(1.7)	13.6	(6.1)	7.8	(2.8)	17.5	(9.4)
2013/14	F	5.0	(1.1)	10.3	(3.3)	7.4	(1.7)	11.1	(6.1)
	S+F	7.5	(2.8)	7.5	(2.8)	10.3	(3.3)	6.8	(2.2)

	P	df	SED^a	SED^b
Season	0.986	1	1.9	
Inoculation	0.498	3	1.8	
Treatment	0.027	2	1.6	
Inoculation*Treatment	0.777	6	3.2	
Inoculation*Season	0.103	3	2.9	2.6
Treatment*Season	0.883	2	2.6	2.3
Inoculation*Treatment*Season	0.612	6	4.7	4.6

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.6 Root rot disease index (%) at GS 31 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis)

Season	**Treatment**	**Control**		**AG 2-1**		**AG 5**		**Rc**	
	UT	5.3	(1.7)	4.5	(1.3)	1.9	(0.4)	9.0	(2.5)
2012/13	F	0.0	(0.0)	5.9	(2.1)	7.1	(2.1)	4.5	(1.3)
	S+F	5.9	(2.1)	0.0	(0.0)	5.3	(1.7)	2.6	(0.8)
	UT	13.7	(6.1)	16.5	(9.4)	13.2	(8.3)	11.1	(5.6)
2013/14	F	20.9	(12.8)	16.0	(7.8)	20.3	(12.2)	22.8	(15.0)
	S+F	16.0	(7.8)	11.2	(5.6)	17.6	(10.0)	18.9	(11.7)

	P	df	SED[a]	SED[b]
Season	0.005	1	2.5	
Inoculation	0.497	3	1.6	
Treatment	0.176	2	1.4	
Inoculation*Treatment	0.315	6	2.8	
Inoculation*Season	0.941	3	3.2	2.3
Treatment*Season	0.046	2	3.0	2.0
Inoculation*Treatment*Season	0.185	6	4.6	4.0

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.7 Root rot disease index (%) at GS 39 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis)

Season	**Treatment**	**Control**		**AG 2-1**		**AG 5**		**Rc**	
	UT	3.7	(0.8)	3.7	(0.8)	6.5	(2.9)	7.5	(2.5)
2012/13	F	2.6	(0.8)	4.5	(1.3)	1.9	(0.4)	0	(0)
	S+F	5.6	(2.1)	1.9	(0.4)	4.5	(1.3)	2.6	(0.8)
	UT	8.5	(3.3)	2.5	(0.6)	6.0	(1.7)	7.8	(2.8)
2013/14	F	3.5	(1.1)	8.5	(2.2)	10.3	(3.3)	9.6	(5)
	S+F	7.0	(2.2)	0	(0)	13.0	(5.0)	6.0	(1.7)

	P	df	SED[a]	SED[b]
Season	0.039	1	1.1	
Inoculation	0.346	3	1.7	
Treatment	0.764	2	1.5	
Inoculation*Treatment	0.376	6	3.0	
Inoculation*Season	0.457	3	2.4	2.4
Treatment*Season	0.268	2	2.1	2.1
Inoculation*Treatment*Season	0.623	6	4.2	4.2

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.8 Root rot disease index (%) at GS 75 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (back-transformed means in parenthesis)

Season	Treatment	Control		AG 2-1		AG 5		Rc	
	UT	10.1	(3.3)	8.5	(2.9)	4.5	(1.3)	9.3	(3.8)
2012/13	F	3.7	(0.8)	6.3	(1.7)	8.8	(3.3)	2.6	(0.8)
	S+F	5.9	(2.1)	4.5	(1.2)	6.3	(1.7)	7.1	(2.1)
	UT	14.2	(6.1)	9.1	(3.9)	11.0	(3.9)	14.1	(6.1)
2013/14	F	8.1	(3.3)	9.5	(2.8)	14.0	(6.1)	6.8	(2.2)
	S+F	12.0	(4.4)	2.5	(0.6)	6.8	(2.2)	12.1	(4.4)

	P	df	SED[a]	SED[b]
Season	0.101	1	1.8	
Inoculation	0.583	3	1.5	
Treatment	0.069	2	1.3	
Inoculation*Treatment	0.038	6	2.7	
Inoculation*Season	0.482	3	2.6	2.2
Treatment*Season	0.763	2	2.4	1.9
Inoculation*Treatment*Season	0.919	6	4.0	3.8

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

5.3.3 Stem base diseases and effects of seed treatments

5.3.3.1 Stem browning

Due to the difficulty in identifying the early symptoms of individual stem base diseases at GS 15, stems were assessed for general stem browning. Figure 5.2 and 5.3 show visual symptoms of stem browning of plants grown from treated and untreated seed in AG 5 and *R. cerealis* inoculated plots. The interaction between pathogen x season was significant (P=0.002) (Table 5.9) showing AG 5 caused significant stem browning compared to the non-inoculated control in 2012/13. In both experiments the highest stem browning at GS 15 occurred in *R. cerealis* inoculated plots. There was also high stem browning in the non-inoculated control in 2012/13 suggesting other pathogens were present. Seed treatments reduced stem browning (P<.001), and the interaction with season was significant (P<.001) (Table 5.9). The interaction was due to fludioxonil not controlling stem browning in the 2013/14 experiment. In contrast, sedaxane + fludioxonil significantly reduced stem browning compared to the untreated by 47% and 38% in 2012/13 and 2013/14 respectively.

5.3.3.2 Stem base diseases

Sharp eyespot, eyespot and BFR were assessed at GS 31, 39 and 75. Data is not presented for eyespot index as there were no significant (P≤0.05) pathogen or treatment effects and there were no interactions. However, eyespot index was significantly higher in 2012/13 than 2013/14 at GS 31, GS 39 and GS 75.

Sharp eyespot index at GS 31 was highest in the *R. cerealis* (32.4%) inoculated plots (P<.001) (Table 5.10). Sharp eyespot symptoms were also observed in AG 2-1 (5.1%) and AG 5 (5%) inoculated plots and the non-inoculated control (4.1%). At GS 31, there was no effect of seed treatments and there were no significant interactions.

The majority of sharp eyespot symptoms at GS 39 in both experiments occurred in *R. cerealis* inoculated plots (P<.001) (Table 5.11). However, there was an AG x season interaction (P=0.005). This was likely due to higher disease assessed in

the non-inoculated plots compared to plots inoculated in AG 2-1. There was no significant (P=<0.05) effect of seed treatment.

Overall at GS 75 the highest sharp eyespot index (27.9%) occurred in *R. cerealis* inoculated plots with low indexes (≤3%) also occurring in the AG 2-1, AG 5 and non-inoculated control plots (Table 5.12). There was no significant (P=<0.05) effect of seed treatment.

BFR index at GS 31 was significantly (P<.001) lower in *R. cerealis* (8.4%) inoculated plots than in AG 2-1 (20.4%) and AG 5 (21.1%) and the non-inoculated control plots (25.8%) (Table 5.13). There was a seed treatment effect (P=0.025) with sedaxane + fludioxonil reducing BFR by 30.4% compared to the untreated.

At GS 39 BFR index in *R. cerealis* (15.1%) inoculated plots was lower than in AG 2-1 (24.9%) and AG 5 (24.4%) and the non-inoculated control (26%) (Table 5.14). BFR was also approximately 23% lower in fludioxonil and sedaxane + fludioxonil treatments than the untreated.

At GS 75 lower BFR in *R. cerealis* (15.5%) inoculated plots was also observed compared to AG 2-1 (27.5%) and AG 5 (27.5%) and the non-inoculated control (23.8%) (Table 5.15). There was no seed treatment effect present at GS 75.

Table 5.9 Stem browning disease index (%) at GS 15 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis)

Season	Treatment	Control		AG 2-1		AG 5		Rc	
2012/13	UT	23	(15.8)	26	(19.6)	37	(35.8)	51	(60.4)
	F	0	(0)	4.6	(2.5)	21	(13.3)	46	(51.4)
	S+F	0	(0)	8.3	(2.9)	12	(9.2)	44	(47.5)
2013/14	UT	3.5	(1.1)	9.9	(3.3)	6	(1.7)	41	(42.2)
	F	5	(1.1)	5	(2.2)	6	(1.7)	42	(45)
	S+F	0	(0)	2.5	(0.6)	0	(0)	36	(34.4)

	P	df	SED^a	SED^b
Season	0.003	1	1.7	
Inoculation	<.001	3	1.9	
Treatment	<.001	2	1.7	
Inoculation*Treatment	0.089	6	3.3	
Inoculation*Season	0.002	3	2.9	2.7
Treatment*Season	<.001	2	2.6	2.4
Inoculation*Treatment*Season	0.39	6	4.8	4.7

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).



Untreated Fludioxonil Sedaxane + Fludioxonil

Figure 5.2 Effect of seed treatment on stem browning of wheat grown in *R. cerealis* inoculated plots



Untreated Fludioxonil Sedaxane + Fludioxonil

Figure 5.3 Effect of seed treatment on stem browning of wheat grown in AG 5 inoculated plots

Table 5.10 Sharp eyespot disease index (%) at GS 31 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis)

Season	Treatment	Control		AG 2-1		AG 5		Rc	
	UT	5.9	(2.2)	8.8	(8.3)	0.0	(0)	30.6	(26.1)
2012/13	F	3.0	(1.1)	2.1	(0.6)	2.1	(0.6)	37.2	(36.7)
	S+F	4.3	(1.1)	3.0	(1.1)	4.9	(2.8)	33.6	(30.6)
	UT	2.9	(0.7)	4.1	(1.5)	2.9	(0.7)	29.9	(25.2)
2013/14	F	2.9	(0.7)	9.8	(4.4)	9.0	(3.7)	32.2	(28.9)
	S+F	5.0	(2.2)	2.9	(0.7)	13.9	(5.9)	29.6	(24.4)

	P	df	SED[a]	SED[b]
Season	0.59	1	1.4	
Inoculation	<.001	3	2.1	
Treatment	0.698	2	1.9	
Inoculation*Treatment	0.393	6	3.7	
Inoculation*Season	0.171	3	3.0	3.1
Treatment*Season	0.584	2	2.6	2.7
Inoculation*Treatment*Season	0.798	6	5.3	5.3

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.11 Sharp eyespot disease index (%) at GS 39 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis)

Season	Treatment	Control		AG 2-1		AG 5		Rc	
	UT	6.1	(2.2)	2.1	(0.6)	0.0	(0)	19.3	(14.4)
2012/13	F	5.2	(1.7)	5.2	(1.7)	3.7	(1.7)	40.7	(42.8)
	S+F	3.0	(1.1)	3.0	(1.1)	7.5	(3.9)	37.1	(36.7)
	UT	6.9	(2.2)	4.1	(1.5)	0.0	(0)	19.6	(11.9)
2013/14	F	8.6	(3.7)	0	(0)	2.9	(0.7)	20.1	(11.9)
	S+F	5.0	(2.2)	4.1	(1.5)	8.1	(3.0)	17.5	(14.1)

	P	df	SED^a	SED^b
Season	0.314	1	2.7	
Inoculation	<.001	3	2.2	
Treatment	0.074	2	1.9	
Inoculation*Treatment	0.12	6	3.8	
Inoculation*Season	0.005	3	3.8	3.2
Treatment*Season	0.223	2	3.5	2.7
Inoculation*Treatment*Season	0.307	6	5.9	5.5

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.12 Sharp eyespot disease index (%) at GS 75 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. Angular transformed disease index (%) (non-transformed means in parenthesis).

Season	Treatment	Control		AG 2-1		AG 5		Rc	
	UT	3.7	(1.7)	2.1	(0.6)	3.0	(1.1)	22.3	(15.0)
2011/12	F	2.1	(0.6)	6.8	(2.8)	2.1	(0.6)	39.6	(41.1)
	S+F	0.0	(0.0)	2.1	(0.6)	7.3	(2.2)	34.3	(33.3)
	UT	10.0	(4.4)	0.0	(0.0)	0.0	(0.0)	29.4	(25.2)
2012/13	F	2.9	(0.7)	0.0	(0.0)	0.0	(0.0)	24.0	(17.8)
	S+F	0.0	(0.0)	0.0	(0.0)	4.1	(1.5)	13.6	(8.2)

	P	df	SED[a]	SED[b]
Season	0.03	1	1.1	
Inoculation	<.001	3	2.2	
Treatment	0.58	2	1.9	
Inoculation*Treatment	0.19	6	3.9	
Inoculation*Season	0.08	3	3.0	3.2
Treatment*Season	0.06	2	2.5	2.8
Inoculation*Treatment*Season	0.17	6	5.4	5.5

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.13 Brown foot rot index (%) at GS 31 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis* in 2012/13 and 2013/14 experiments. Angular transformed disease index (%) (non-transformed means in parenthesis).

Season	**Treatment**	**Control**		**AG 2-1**		**AG 5**		**Rc**	
	UT	29.9	(26.3)	27	(22.5)	22.9	(17.1)	11.3	(6.0)
2012/13	F	23.6	(17.5)	19.3	(12.1)	21.6	(16.8)	8.1	(3.5)
	S+F	23.9	(17.5)	15	(11.1)	18.8	(13.3)	5.8	(2.9)
	UT	24	(18.5)	17.1	(9.6)	19.4	(11.1)	6.9	(2.2)
2013/14	F	16.5	(8.9)	15.2	(8.1)	11.9	(4.4)	9	(3.7)
	S+F	19.5	(11.9)	5.7	(1.5)	12.7	(8.9)	2.9	(0.7)

	P	df	SED^a	SED^b
Season	0.012	1	2.49	
Inoculation	<.001	3	2.90	
Treatment	0.025	2	2.51	
Inoculation*Treatment	0.923	6	5.03	
Inoculation*Season	0.386	3	4.37	4.15
Treatment*Season	0.948	2	3.84	3.59
Inoculation*Treatment*Season	0.752	6	7.31	7.18

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.14 Brown foot rot index (%) at GS 39 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis* in 2012/13 and 2013/14 experiments. Angular transformed disease index (%) (non-transformed means in parenthesis).

Season	Treatment	Control		AG 2-1		AG 5		Rc	
	UT	29.0	(25.1)	30.3	(26.4)	28.7	(23.2)	18.7	(11.8)
2012/13	F	23.2	(16.2)	26.4	(21.3)	21.1	(14.0)	10.5	(4.1)
	S+F	25.8	(20.6)	17.9	(12.4)	23.5	(16.5)	16.1	(8.3)
	UT	23.1	(17.8)	23.7	(17.0)	29.5	(24.4)	11.9	(4.4)
2013/14	F	23.3	(17.0)	21.6	(14.8)	19.4	(11.1)	13.6	(5.9)
	S+F	17.1	(9.6)	12.7	(5.2)	18.0	(9.6)	18.3	(10.4)

	P	df	SED^a	SED^b
Season	0.049	1	2.20	
Inoculation	<.001	3	2.09	
Treatment	0.001	2	1.81	
Inoculation*Treatment	0.165	6	3.62	
Inoculation*Season	0.135	3	3.39	2.98
Treatment*Season	0.142	2	3.05	2.58
Inoculation*Treatment*Season	0.148	6	5.41	5.17

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.15 Brown foot rot index (%) at GS 75 of wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis* in 2012/13 and 2013/14 experiments. Angular transformed disease index (%) (non-transformed means in parenthesis).

Season	Treatment	Control		AG 2-1		AG 5		Rc	
2012/13	UT	29.6	(25.1)	30.5	(26.3)	26.6	(23.2)	17.2	(10.5)
	F	27.3	(22.5)	25.4	(19.4)	27.4	(21.6)	14.2	(7.6)
	S+F	28.7	(23.8)	26.6	(21.0)	28.5	(23.2)	15.3	(7.9)
2013/14	UT	23.0	(15.6)	29.7	(25.2)	26.5	(20.0)	14.8	(9.6)
	F	22.6	(15.6)	24.8	(19.3)	25.5	(19.3)	9.4	(4.4)
	S+F	25.0	(18.5)	23.0	(16.3)	26.0	(20.0)	18.3	(11.1)

	P	df	SED^a	SED^b
Season	0.149	1	2.45	
Inoculation	<.001	3	2.43	
Treatment	0.529	2	2.10	
Inoculation*Treatment	0.969	6	4.21	
Inoculation*Season	0.514	3	3.88	3.47
Treatment*Season	0.858	2	3.47	3.01
Inoculation*Treatment*Season	0.739	6	6.26	6.01

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

5.3.4 Pathogen DNA in soil at GS 15

Pathogen DNA in soil samples was quantified only at GS 15. Overall the highest DNA concentrations were those of *R. cerealis* (467.7 pg ng^{-1} of soil) followed by AG 2-1 (85.31 pg ng^{-1} of soil) then AG 5(4.68 pg ng^{-1} of soil) (Table 5.16).

5.3.5 Effect of inoculation and seed treatment *on Rhizoctonia* DNA in roots

DNA of AG 2-1, AG 5 and *R. cerealis* was detected in the roots at GS 31 and GS 75. DNA extractions were not performed on GS 39 samples.

At GS 31 there were differences in the amount of *Rhizoctonia* DNA in the roots (P=0.012), and the interaction with season was significant (P=0.049) (Table 5.17). This is due to the differences in the amount of AG 2-1 and *R. cerealis* between seasons. Overall in 2012/13 the highest DNA concentrations were those of AG 2-1 but in the following experiment *R. cerealis* had the highest DNA levels in roots. There was no effect of seed treatment although overall at GS 31 in both experiments the lowest DNA concentrations occurred in sedaxane + fludioxonil treatments.

At GS 75 DNA concentrations for AG 2-1 and *R. cerealis* were considerably lower than at the previous growth stage but relatively similar for AG 5 (Table 5.18). There was an inoculation x season interaction (P=0.001). This shows that sedaxane + fludioxonil treatments were not very effective against AG 5 in both seasons. Despite this AG 2-1 and *R. cerealis* DNA in roots was reduced by sedaxane + fludioxonil compared to the untreated.

5.3.6 Effect of inoculation and seed treatment *on Rhizoctonia* DNA in stems

At GS 15 *Rhizoctonia* spp. DNA in stems was effected by inoculation (P<.001) and seed treatment (P=0.013), there were also AG x season (P<.001) and treatment x season (P=0.048) interactions (Table 5.19). However, the three way interaction was also significant (P=0.04) but there was no clear explanation for the three way interaction. That said, overall the amount of *Rhizoctonia* spp. DNA in stems was lower in sedaxane + fludioxonil treatments in both experiments compared to the untreated.

At GS 31 the amount of AG 2-1 and *R. cerealis* DNA in stems was 4 and 12.5 fold higher than AG 5 respectively (P=0.002) (Table 5.20). However, there was no effect of seed treatment and there were no interactions at GS 31. In both seasons at GS 31 the lowest DNA concentrations occurred in the sedaxane + fludioxonil treatments.

The highest DNA levels in stems at GS 75 were those of *R. cerealis* which were >400 and 22 fold higher than AG 2-1 or AG 5 respectively (P<.001) (Table 5.21). Seed treatment had no significant effect on pathogen DNA in stems and there were no interactions. However, overall DNA concentrations were lower in sedaxane + fludioxonil and the untreated compared to fludioxonil.

5.3.7 Effect of inoculation and seed treatment *on Microdochium* spp. DNA in stems

Low DNA levels of *M. nivale* and *M. majus* were detected at GS 15 (Table 5.22 and 5.23). There was no pathogen or treatment effect for *M. nivale* but the lowest DNA concentrations were found in plants grown from fludioxonil and sedaxane + fludioxonil treated seed (Table 5.22). There was a significant (P<.001) treatment effect on *M. majus* DNA in stems at GS 15 with significantly lower concentrations in fludioxonil and sedaxane + fludioxonil treatments compared to the untreated. However, only sedaxane + fludioxonil showed total control of *M. majus* in all inoculated plots.

Table 5.16 *Rhizoctonia* DNA (pg g^{-1} of soil) in soil collected at GS 15 of wheat plants grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis

Season	Treatment	AG 2-1		AG 5		Rc	
	UT	2.47	(295.1)	2.15	(141.3)	3.62	(4168.7)
2012/13	F	2.16	(144.5)	0.81	(6.46)	1.65	(44.7)
	S+F	2.95	(891.3)	1.67	(46.8)	3.44	(2754.2)
	UT	2.35	(223.9)	-1.02	(0.10)	3.65	(4466.8)
2013/14	F	1.49	(30.9)	0.17	(1.48)	0.63	(4.27)
	S+F	-0.41	(0.39)	-0.63	(0.23)	2.77	(588.8)

	P	df	SED[a]	SED[b]
Season	0.091	1	0.63	
Inoculation	0.013	2	0.65	
Treatment	0.268	2	0.65	
Inoculation*Treatment	0.541	4	1.13	
Inoculation*Season	0.533	2	0.99	0.93
Treatment*Season	0.575	2	0.99	0.93
Inoculation*Treatment*Season	0.614	4	1.61	1.61

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.17 *Rhizoctonia* DNA (pg ng^{-1} of total DNA) in wheat roots at GS 31 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis

Season	Treatment	AG 2-1		AG 5		Rc	
	UT	-0.82	(0.151)	-1.68	(0.021)	-1.24	(0.058)
2012/13	F	-1.06	(0.087)	-1.39	(0.041)	-0.76	(0.174)
	S+F	-0.72	(0.191)	-1.42	(0.038)	-2.19	(0.006)
	UT	-0.46	(0.347)	-2.00	(0.010)	-0.49	(0.324)
2013/14	F	-0.95	(0.112)	-2.69	(0.002)	-0.46	(0.347)
	S+F	-2.06	(0.009)	-1.94	(0.011)	-1.19	(0.065)

	P	df	SED^a	SED^b
Season	0.834	1	0.475	
Inoculation	0.012	2	0.278	
Treatment	0.256	2	0.278	
Inoculation*Treatment	0.281	4	0.482	
Inoculation*Season	0.049	2	0.575	0.398
Treatment*Season	0.532	2	0.575	0.398
Inoculation*Treatment*Season	0.43	4	0.805	0.689

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.18 *Rhizoctonia* DNA (pg ng^{-1} of total DNA) in wheat roots at GS 75 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis

Season	Treatment	AG 2-1		AG 5		Rc	
	UT	-2.91	(0.001)	-1.56	(0.028)	-2.09	(0.008)
2012/13	F	-3.32	(0.0005)	-1.28	(0.053)	-1.82	(0.015)
	S+F	-3.31	(0.0005)	-0.96	(0.110)	-2.85	(0.001)
	UT	-2.06	(0.009)	-2.68	(0.002)	-1.11	(0.078)
2013/14	F	-1.8	(0.016)	-3.18	(0.0007)	-1.66	(0.022)
	S+F	-3.66	(0.0002)	-1.65	(0.022)	-2.31	(0.005)

	P	df	SED^a	SED^b
Season	0.993	1	0.265	
Inoculation	<.001	2	0.261	
Treatment	0.364	2	0.261	
Inoculation*Treatment	0.025	4	0.452	
Inoculation*Season	0.001	2	0.404	0.373
Treatment*Season	0.728	2	0.404	0.373
Inoculation*Treatment*Season	0.206	4	0.665	0.647

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.19 *Rhizoctonia* DNA (pg ng^{-1} of total DNA) in wheat stems at GS 15 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis

Season	Treatment	AG 2-1		AG 5		Rc	
	UT	-2.955	(0.001)	-1.744	(0.018)	-0.849	(0.142)
2012/13	F	-3.551	(0.0003)	-3.149	(0.001)	-1.111	(0.077)
	S+F	-2.852	(0.001)	-3.584	(0.0003)	-0.826	(0.149)
	UT	-2.485	(0.003)	-3.691	(0.0002)	-0.153	(0.703)
2013/14	F	-2.094	(0.008)	-3.164	(0.001)	-0.812	(0.154)
	S+F	-2.719	(0.002)	-4	(0)	-1.142	(0.072)

	P	df	SED^a	SED^b
Season	0.831	1	0.179	
Inoculation	<.001	2	0.181	
Treatment	0.013	2	0.181	
Inoculation*Treatment	0.141	4	0.313	
Inoculation*Season	<.001	2	0.277	0.259
Treatment*Season	0.048	2	0.277	0.259
Inoculation*Treatment*Season	0.04	4	0.459	0.448

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.20 *Rhizoctonia* DNA (pg ng^{-1} of total DNA) in wheat stems at GS 31 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis

Season	Treatment	AG 2-1		AG 5		Rc	
	UT	-0.46	(0.35)	-0.86	(0.14)	-0.79	(0.16)
2012/13	F	-1.54	(0.03)	-0.83	(0.15)	0.18	(1.51)
	S+F	-0.20	(0.63)	-1.64	(0.02)	-0.57	(0.27)
	UT	-0.64	(0.23)	-1.78	(0.02)	0.08	(1.20)
2013/14	F	-0.57	(0.27)	-2.14	(0.01)	0.23	(1.70)
	S+F	-1.40	(0.04)	-1.44	(0.04)	-0.86	(0.14)

	P	df	SED[a]	SED[b]
Season	0.70	1	0.493	
Inoculation	0.002	2	0.285	
Treatment	0.642	2	0.285	
Inoculation*Treatment	0.371	4	0.494	
Inoculation*Season	0.312	2	0.595	0.407
Treatment*Season	0.790	2	0.595	0.407
Inoculation*Treatment*Season	0.106	4	0.828	0.705

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.21 *Rhizoctonia* DNA (pg ng^{-1} of total DNA) in wheat stems at GS 75 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *Rhizoctonia* DNA values are log_{10} transformed with back-transformed means in parenthesis

Season	**Treatment**	**AG 2-1**		**AG 5**		**Rc**	
	UT	-2.41	(0.004)	-0.52	(0.302)	0.17	(1.48)
2012/13	F	-0.89	(0.129)	-0.07	(0.851)	0.95	(8.91)
	S+F	-1.64	(0.023)	-0.51	(0.309)	0.63	(4.27)
	UT	-2.93	(0.001)	-2.3	(0.005)	0.22	(1.66)
2013/14	F	-3.48	(0.0003)	-2.4	(0.004)	0.25	(1.78)
	S+F	-4	(0)	-1.5	(0.032)	-1.13	(0.074)

	P	df	SED^a	SED^b
Season	0.003	1	0.277	
Inoculation	<.001	2	0.280	
Treatment	0.218	2	0.280	
Inoculation*Treatment	0.691	4	0.485	
Inoculation*Season	0.162	2	0.428	0.400
Treatment*Season	0.114	2	0.428	0.400
Inoculation*Treatment*Season	0.212	4	0.709	0.693

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.22 *M. nivale* DNA (pg ng^{-1} of total DNA) in wheat stems at GS 15 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*. *M. nivale* DNA values are log_{10} transformed with back-transformed means in parenthesis

Season	**Treatment**	**AG 2-1**		**AG 5**		**Rc**	
	UT	-2.00	(0.01)	-2.56	(0.003)	-2.07	(0.009)
2012/13	F	-2.94	(0.001)	-2.66	(0.002)	-3.01	(0.0010)
	S+F	-3.01	(0.001)	-3.52	(0.0003)	-2.64	(0.0023)
	UT	-4.00	(0)	-4.00	(0)	-4.00	(0)
2013/14	F	-4.00	(0)	-4.00	(0)	-4.00	(0)
	S+F	-4.00	(0)	-3.30	(0.001)	-4.00	(0)

	P	df	SED^a	SED^b
Season	0.012	1	0.314	
Inoculation	0.957	3	0.325	
Treatment	0.413	2	0.325	
Inoculation*Treatment	0.96	6	0.563	
Inoculation*Season	0.641	3	0.493	0.465
Treatment*Season	0.268	2	0.493	0.465
Inoculation*Treatment*Season	0.809	6	0.821	0.805

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.23 *M. majus* DNA (pg ng^{-1} of total DNA) in wheat stems at GS 15 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis. M. majus* DNA values are log_{10} transformed with back-transformed means in parenthesis

Season	**Treatment**	**AG 2-1**		**AG 5**		**Rc**	
	UT	-2.38	(0.004)	-2.59	(0.003)	-2.62	(0.002)
2012/13	F	-3.79	(0.0002)	-4.00	(0)	-4.00	(0)
	S+F	-4.00	(0)	-4.00	(0)	-4.00	(0)
	UT	-4.00	(0)	-3.12	(0.001)	-2.58	(0.003)
2013/14	F	-4.00	(0)	-4.00	(0)	-4.00	(0)
	S+F	-4.00	(0)	-4.00	(0)	-4.00	(0)

	P	df	SED^a	SED^b
Season	0.118	1	0.137	
Inoculation	0.799	3	0.177	
Treatment	<.001	2	0.177	
Inoculation*Treatment	0.713	6	0.306	
Inoculation*Season	0.216	3	0.247	0.252
Treatment*Season	0.107	2	0.247	0.252
Inoculation*Treatment*Season	0.372	6	0.434	0.437

SED - standard error of a difference. SED^a - for comparing means between seasons. SED^b - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

5.3.8 Physiological assessments

The spectrometer assessments conducted at GS 39, GS 75 and GS 85 enabled a range of spectral reflectance indices (SRI) to be calculated (Table 5.2).

The photochemical reflectance index (PRI) had a time x AG x treatment interaction (P=0.05) (Figure 5.4). This was shown at GS 85 in *R. cerealis* inoculated plots were PRI was 23.6% and 32.9% higher in fludioxonil and sedaxane + fludioxonil treatments than the untreated, respectively. PRI was also 20.3% higher in the fludioxonil treatment compared to the untreated in the non-inoculated control plots. However, in AG 5 inoculated plots PRI was 18.4% higher in the untreated than in fludioxonil treatments. There was also an interaction between time x treatment for PRI (P=0.028) (Figure 5.5). This showed that overall PRI at GS 39 was significantly lower in sedaxane + fludioxonil than the untreated by 3.5%. However, at GS 85 the reverse was shown with PRI 9.3% higher in sedaxane + fludioxonil than the untreated.

There was a time x treatment interaction for Normalized Water Index 2 (NWI-2) (P<0.10) (Figure 5.6). This was probably due to the NWI-2 at GS 39 been 2.3 and 5.9% lower in fludioxonil and sedaxane + fludioxonil treatments compared to the untreated respectively. However, this effect was reversed at GS 75 with NWI-2 higher in sedaxane + fludioxonil treatments than the untreated.

There was also a time x treatment interaction for ratio analysis of reflectance spectra carotenoid (RARSc) (P<0.10) (Figure 5.7). The main effect was shown at GS 39 when RARSc was 6.2% higher in sedaxane + fludioxonil treatments than the untreated. At GS 75 and GS 85 there was no difference between treatments.

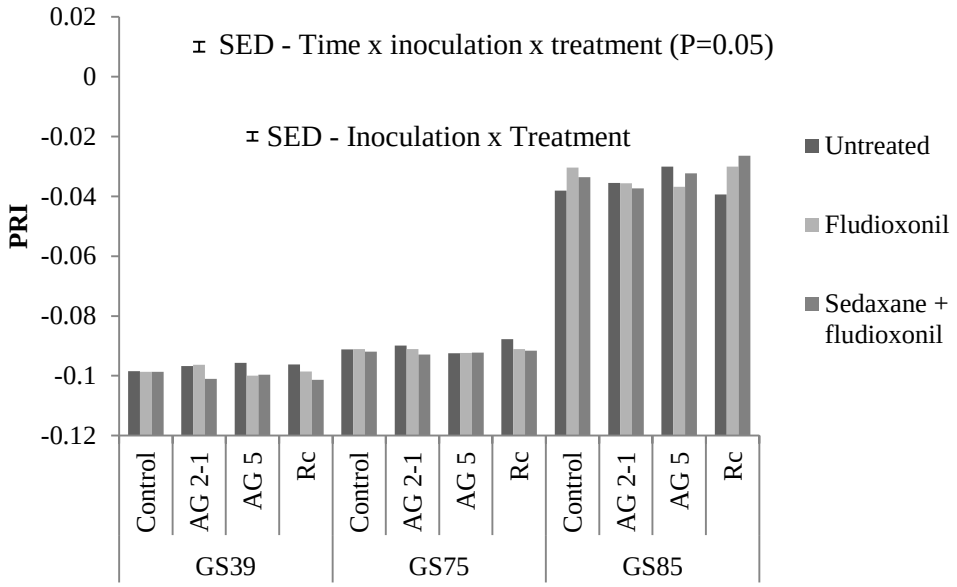


Figure 5.4 Photochemical reflectance index (PRI) for wheat plants at GS 39, GS 75 and GS 85 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis.*

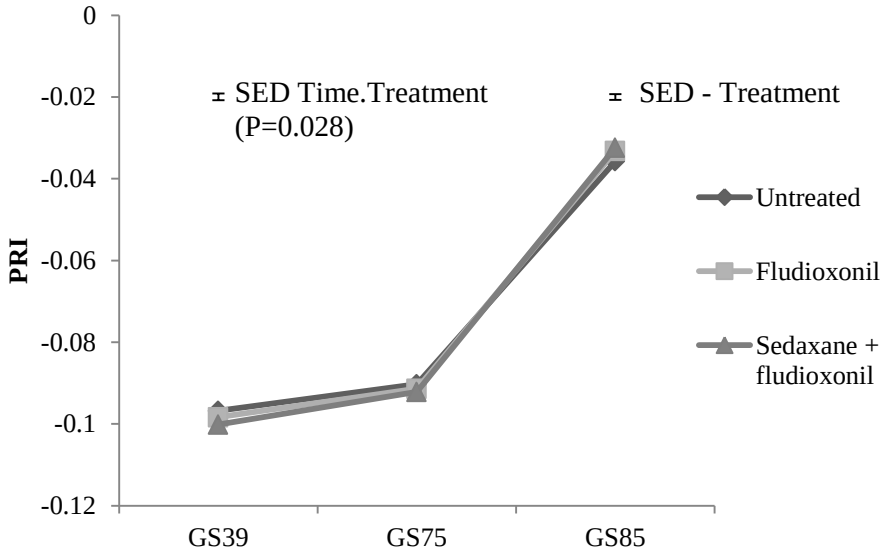


Figure 5.5 Photochemical reflectance index (PRI) for wheat plants at GS 39, GS 75 and GS 85 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots.

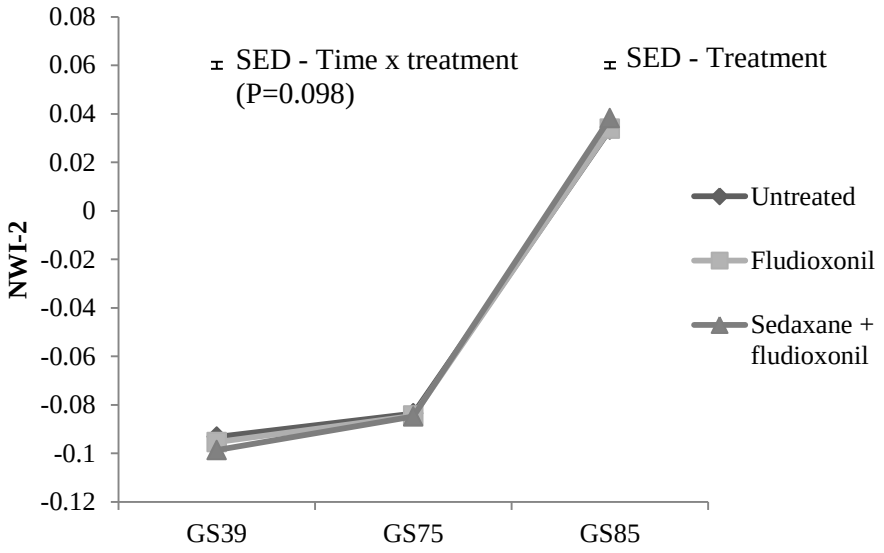


Figure 5.6 Normalized water index 2 (NWI-2) for wheat plants at GS 39, GS 75 and GS 85 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots.

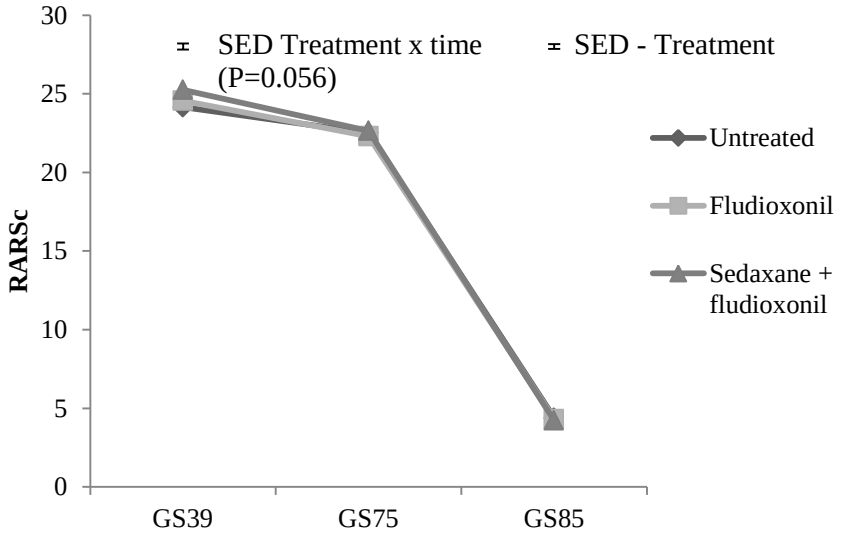


Figure 5.7 Ratio analysis of reflectance spectra carotenoid (RARSc) for wheat plants at GS 39, GS 75 and GS 85 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots.

5.3.9 Yield components

5.3.9.1 Plant height (mm) (GS 15)

There was a treatment x season interaction for plant height at GS 15 (Table 5.24). Plant height was increased consistently by fludioxonil and sedaxane + fludioxonil in AG 2-1 and *R. cerealis* inoculated plots in both experiments. The effect of seed treatments on plants in AG 5 inoculated plots was inconsistent between experiments. Inoculation was also significant (P=0.045) (Table 5.24). Plants grown in *R. cerealis* (137.5 mm), AG 2-1 (139.8 mm) and AG 5 (141.1 mm) inoculated plots were shorter than those in the non-inoculated (142.7 mm) controls.

5.3.9.2 Canopy green area (cm^2) (GS 15)

There was no difference in green area between inoculated and non-inoculated control plots (Table 5.25). The effect of seed treatment was significant (P<0.001), and there was a treatment x season interaction (P=0.012). Green area was lowest in *R. cerealis* inoculated plots and this is where the most consistent response to seed treatments occurred. Fludioxonil treatments were more effective in 2012/13 but there was greater response to sedaxane + fludioxonil in 2013/14.

5.3.9.3 Above ground dry matter (AGDM) (g m^2)

At GS 39 and GS 75 there was no effect of pathogen or seed treatment on AGDM and the interactions were not significant (P≤0.05) (Appendix 8). There were differences between the two experiments at GS 39 when in 2012/13 AGDM was lower than 2013/14 (P=0.007).

5.3.9.4 Green area index (GAI)

The green area index (GAI defined as the green canopy area per unit ground area) at GS 39 and GS 75 was not affectedededededededededededed by pathogen or seed treatment and there were no interactions (Appendix). There were differences between the two experiments at GS 39 and GS 75 with higher GAI's in 2013/14 than 2012/13 (Appendix 9).

5.3.9.5 Grain yield components

Ear partitioning index (EPI) is the fraction of above-ground DM in the ear. At GS 75 there was an AG x season interaction (P=0.048) (Table 5.26). This showed in 2012/13 there was no difference in EPI between the inoculated and non-inoculated control plots. However, in 2013/14 that AG 5 (0.35) and *R. cerealis* (0.33) inoculated plots had significantly lower EPI than AG 2-1 (0.37) but not compared to the control (0.36). There was an AG x treatment x season interaction (P<0.10) likely due to the lower EPI from untreated seed in *R. cerealis* inoculated plots in 2013/14.

Grain yield was significantly (P=0.023) reduced in *R. cerealis* inoculated plots compared to AG 2-1, AG 5 and the non-inoculated control by 6.2%, 8.3% and 8.5% respectively (Table 5.26). However, there was no significant effect of seed treatment on yield and there were no interactions.

There was also no difference in SPW between the inoculated and non-inoculated control plots or from either seed treatment and there were no interactions. However, TGW was significantly higher in AG 5 (40.48) and *R. cerealis* (40.69) inoculated plots than the non-inoculated control (39.39) (Table 5.26). However, seed treatment did not affect TGW and the interactions were not significant.

Table 5.24 Plant height (mm) of wheat at GS 15 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*

Season	Treatment	Control	AG 2-1	AG 5	Rc
2012/13	UT	151.8	147.6	152.2	146.1
	F	162.6	164.2	159.1	165.2
	S+F	158.7	163.7	164.5	156.6
2013/14	UT	121.3	110.1	118.2	106.2
	F	124.3	116.4	117.8	113.4
	S+F	122.7	117.6	117.5	118.8

	P	df	SED[a]	SED[b]
Season	<.001	1	2.44	
Inoculation	0.045	3	1.85	
Treatment	<.001	2	1.60	
Inoculation*Treatment	0.24	6	3.20	
Inoculation*Season	0.081	3	3.35	2.64
Treatment*Season	0.019	2	3.08	2.29
Inoculation*Treatment*Season	0.515	6	5.02	4.24

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.25 Green area (cm^2) of wheat at GS 15 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1 and AG 5 and *R. cerealis*

Season	Treatment	Control	AG 2-1	AG 5	Rc
	UT	90.1	96.9	100.8	85.7
2012/13	F	120.2	110.1	116.1	133.1
	S+F	97.9	105.3	106.7	109.5
	UT	62.6	49.3	66.4	48
2013/14	F	59.9	52.8	67.8	56.6
	S+F	54.2	48.0	54.8	70.1

	P	df	SED[a]	SED[b]
Season	<.001	1	6.9	
Inoculation	0.298	3	4.4	
Treatment	<.001	2	3.8	
Inoculation*Treatment	0.223	6	7.6	
Inoculation*Season	0.605	3	8.8	6.3
Treatment*Season	0.012	2	8.2	5.5
Inoculation*Treatment*Season	0.546	6	12.5	10.9

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc). UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}).

Table 5.26 Harvest yield components for ear portioning index, grain yield (t ha^{-1}), specific weight (SPW), thousand grain weight (TGW) for wheat grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*.

	Ear partitioning index			**Yield (t ha^{-1})**			**SPW (kg/hl)**			**TGW (g)**		
	2012/13	2013/14	Mean	2012/13	2013/14	Mean	2012/13	2013/14	Mean	2012/13	2013/14	Mean
Control	0.362	0.360	0.361	11.01	17.59	13.83	71.85	72.14	71.98	41.04	40.23	40.69
AG 2-1	0.355	0.373	0.362	10.57	17.38	13.49	72.17	72.28	72.21	41.03	40.05	40.61
AG 5	0.353	0.350	0.352	10.79	17.82	13.80	71.88	71.62	71.77	41.00	39.79	40.48
Rc	0.355	0.327	0.343	10.18	15.94	12.65	72.03	70.74	71.48	40.54	37.85	39.39
	P	SED[a]	SED[b]	P	SED[a]	SED[b]	P	SED[a]	SED[b]	P	SED[a]	SED[b]
Season	0.902	0.027		<.001	0.518		0.457	0.355		0.093	0.689	
Inoculation	0.06	0.007		0.023	0.421		0.501	0.493		0.044	0.508	
Treatment	0.565	0.007		0.554	0.365		0.276	0.427		0.581	0.440	
Inoculation*Treatment	0.383	0.014		0.595	0.729		0.602	0.854		0.567	0.880	
Inoculation*season	0.048	0.029	0.011	0.469	0.735	0.602	0.396	0.706	0.705	0.250	0.933	0.726
Treatment*Season	0.567	0.028	0.010	0.834	0.670	0.521	0.464	0.612	0.610	0.671	0.859	0.629
Inoculation *Treatment*Season	0.063	0.033	0.019	0.644	1.124	1.042	0.394	1.221	1.221	0.551	1.387	1.257

SED - standard error of a difference. SED[a] - for comparing means between seasons. SED[b] - for comparing means within seasons. df – degrees of freedom. *R. solani* AG 2-1 (AG 2-1), *R. solani* AG 5 (AG 5), *R. cerealis* (Rc).

5.3.10 Relationships between disease indexes and pathogen DNA

Regression analyses (R^2) using disease indexes and pathogen DNA revealed there were no significant correlations between *Rhizoctonia* DNA in roots and root rot (data not shown). There were significant but generally weak to moderate (R^2 = ≤0.40) relationships between *Rhizoctonia* spp. DNA in stems and symptoms on the stems (Table 5.27). The strongest relationship (0.60) was between *Rhizoctonia* spp. DNA in stems at GS 15 and stem browning index at GS 15. There was also a good correlation (0.45) between *Rhizoctonia* spp. DNA in stems at GS 15 and sharp eyespot index at GS 75. The relationship between *Rhizoctonia* spp. DNA in stems at GS75 and sharp eyespot index at the same GS was also good (0.43). Sharp eyespot also showed negative correlations with BFR at GS 31, GS 39 and GS 75 (Table 5.27). There was also a very weak but significant correlation between stem browning at GS 13 and *Microdochium* DNA in stems at GS 15.

Regression analysis for grain yield with root rot, sharp eyespot and BFR were generally weak with the best, but positive, correlation found with root rot at GS 31 (0.40) (Table 5.28). Yield and DNA showed the best correlation with *Rhizoctonia* spp. DNA in stems at GS 75 (0.26). Yield had the strongest correlations with eyespot index especially at GS 31 (0.44).

5.3.11 Multiple linear regression of yield components with plant developmental assessments, root and stem disease indexes and spectral reflectance indices

Multiple linear regression (MLR) was used to account for variation in the yield components of the 2012/13 and 2013/14 field experiments using plant developmental assessments, root and stem disease indexes and spectral reflectance indices (Table 5.29). MLR accounted for 90% of the variation in plant height at GS 13 (P<.001) and showed a negative relationship to season and stem browning index at GS 15 but was positively related to seed treatment. Green area (GA) at GS 15 was not significantly related to any of the independent variables except with season (negative) which accounted for 65% of the variation (P<.001).

The MLR model for AGDM at GS 39 accounted for only 18% of the variation in the data set (P<.001) and was positively related to season and G-NDVI and negatively related to NWI-3.

Green area index at GS75 was positively related to season and G-NDVI but negatively related to plant length at GS 13 this MRL model accounted for 72% of the variation (P<.001). The MRL model for AGDM at GS 75 was weak accounting for just 12% of the variation (P=0.004), this revealed a negative relationship with PRI and the percentage of seedlings emerged four weeks after sowing but was positively related to G-NDVI. Harvest yield components at GS 92 revealed EPI was negatively related to root rot index (GS 15), sharp eyespot (GS 31) and G-NDVI (GS 39) (P<.001), accounting for just 15% of the variation.

The MRL model revealed grain yield (t ha^{-1}) was positively related to season, the percentage of seedlings emerged four weeks after sowing and G-NDVI (GS39), and negatively related to *Rhizoctonia* spp., (pathogen) and NWI-1 (GS 39) (P<.001) which accounted for 91% of the variation. Thousand grain weight MRL model accounted for just 15% of the variation (P=0.001) with season and G-NDVI positively related, and *Rhizoctonia* spp. (AG) and NWI-1 negatively related.

Table 5.27 Summary of significant regressions for stem base diseases and *log*$_{10}$ DNA of *Rhizoctonia* spp. in stems for 2012/13 and 2013/14 field experiments

Response variate (y)	Independent variate (x)	Equation	R^2	P
Stem browning index at GS 15	*log*$_{10}$ DNA of *Rhizoctonia* in stems at GS 15	y = 52.3 + 13.5x	0.60	<.001
Sharp eyespot index at GS 31	*log*$_{10}$ DNA of *Rhizoctonia* in stems at GS 15	y = 31.0 + 7.40x	0.40	<.001
Sharp eyespot index at GS 39	*log*$_{10}$ DNA of *Rhizoctonia* in stems at GS 15	y = 25.4 + 6.28x	0.30	<.001
Sharp eyespot index at GS 75	*log*$_{10}$ DNA of *Rhizoctonia* in stems at GS 15	y = 28.8 + 7.81x	0.45	<.001
Sharp eyespot index at GS 75	*log*$_{10}$ DNA of *Rhizoctonia* in stems at GS 31	y = 14.1 + 3.89x	0.07	0.02
Sharp eyespot index at GS 75	*log*$_{10}$ DNA of *Rhizoctonia* in stems at GS 75	y = 17.8 + 6.20x	0.43	<.001
log$_{10}$ DNA *Rhizoctonia* in stems at GS 31	Stem browning index at GS 15	y = 0.11 - 0.31x	0.11	0.005
log$_{10}$ DNA *Rhizoctonia* in stems at GS 75	Stem browning index at GS 15	y = 0.18 - 0.57x	0.19	<.001
log$_{10}$ DNA *Rhizoctonia* in stems at GS 75	Sharp eyespot index at GS 31	y = 1.84 - 0.05x	0.21	<.001
log$_{10}$ DNA *Rhizoctonia* in stems at GS 75	Sharp eyespot index at GS 39	y = 1.80 - 0.06x	0.31	<.001
Stem browning index at GS 15	*log*$_{10}$ DNA of *Microdochium* in stems at GS 15	y = 28.68 + 3.43x	0.04	0.043
Sharp eyespot index at GS 31	BFR index GS 31	y = 21.57 – 0.53x	0.20	<.001
Sharp eyespot index at GS 39	BFR index GS 39	y = 24.86 – 0.67x	0.23	<.001
Sharp eyespot index at GS 75	BFR index GS 75	y = 27.4 – 0.74x	0.26	<.001

Note –Regressions with DNA data do not include data from non-inoculated control plots as no DNA extractions were performed.

Table 5.28 Summary of significant regressions for yield (t ha^{-1}), root and stem diseases and *log*$_{10}$ DNA of *Rhizoctonia* spp. for the 2012/13 and 2013/14 field experiments

Response variate (y)	Independent variate (x)	Equation	R^2	P
Yield (t ha^{-1})	Stem browning GS 15	y = 14.60 - 0.06x	0.09	0.003
Yield (t ha^{-1})	Root rot GS 31	y = 10.90 + 0.27x	0.40	<.001
Yield (t ha^{-1})	Root rot GS 39	y = 12.70 + 0.15x	0.05	0.027
Yield (t ha^{-1})	Root rot GS 75	y = 12.32 + 0.14x	0.04	0.044
Yield (t ha^{-1})	Sharp eyespot GS 75	y = 14.00 - 0.06x	0.04	0.045
Yield (t ha^{-1})	*log*$_{10}$ DNA of *Rhizoctonia* in soil at GS 15	y = 14.30 - 0.57x	0.12	0.003
Yield (t ha^{-1})	*log*$_{10}$ DNA of *Rhizoctonia* in stems at GS 75	y = 12.14 - 1.13x	0.26	<.001
Yield (t ha^{-1})	BFR index GS 31	y = 15.42 - 0.10x	0.12	<.001
Yield (t ha^{-1})	BFR index GS 39	y = 15.63 - 0.10x	0.06	0.017
Yield (t ha^{-1})	Eyespot index GS 31	y = 15.60 - 0.22x	0.44	<.001
Yield (t ha^{-1})	Eyespot index GS 39	y = 15.66 - 0.22x	0.31	<.001
Yield (t ha^{-1})	Eyespot index GS 75	y = 15.66 - 0.15x	0.34	<.001

Note –Regressions with DNA data do not include data from non-inoculated control plots as no DNA extractions were performed.

Table 5.29 Multiple linear regression models of the 2012/13 and 2013/14 field experiments accounting for variation in yield components using plant developmental assessments, root and stem disease indexes and spectral reflectance indices at GS 15, GS 31, GS 39, GS 75 and GS 85.

Response variate	GS	Factors and independent variables in the model	R^2	P
Plant height (mm)	13	195.9 - 42 (Season) + 3.5 (treat) - 0.14 (SB GS15)	0.90	<.001
GA (cm^2)	13	154.9 - 48.5 (Season)	0.65	<.001
GAI	39	-36.6 + 2.78 (Season) + 0.13 (treat) - 10.76 (WI GS39) + 50.7 (R-NDVI GS39)	0.74	<.001
AGDM (g m^2)	39	-1814 + 143.5 (Season) -3476 (NWI-3 GS39) + 2344 (G-NDVI GS39)	0.18	<.001
GAI	75	-32.9 + 0.67 (Season) - 0.02 (P/LN GS15) + 47.4 (G-NDVI GS39)	0.72	<.001
AGDM (g m^2)	75	2995 - 2.77 (Emergence) + 4928 (G-NDVI GS39) - 2509 (PRI GS75)	0.12	0.004
EPI	92	1.03 - 0.002 (RR GS 15) - 0.0006 (SE GS 31) - 0.78 (G-NDVI GS39)	0.15	0.001
Yield (t ha^{-1})	92	-42.1 + 9.34 (Season) - 0.24 (AG) + 0.02 (Emergence) + 44.3 (G-NDVI GS39) - 61.1 (NWI-1 GS39)	0.91	<.001
TGW	92	6.3 + 2.2 (Season) - 0.38 (AG) + 32.2 (G-NDVI GS 39) -70.2 (NWI-1 GS39)	0.15	0.001

SB – Stem browning index, Treat –seed treatment, WI – water index, R-NDVI - Red normalized difference vegetation index, NWI-3 - Normalized water index 3, G-NDVI - Green normalized difference vegetation index, P/LN – plant length (mm), Emergence (4 weeks after sowing), PRI - Photochemical reflectance index, RR- root rot, SE – sharp eyespot, NWI-1 - Normalized water index 1, NDVI - Normalized difference vegetation index. GA – Green area, AGDM – Above ground dry matter, GAI – Green area index, EPI – Ear partitioning index, TGW –Thousand grain weight. * - *Rhizoctonia* spp. DNA

5.3.12 Meteorological data for 2012/13 and 2013/14 field experiments

Meteorological data was gathered from the University of Nottingham meteorological station, situated adjacent to the experimental fields. In the period from sowing to GS 15 conditions were wetter with total rainfall was 123% greater and also warmer with mean temperature 0.8°C higher in 2012/13 than 2013/14 (Table 5.30). However, from GS 13 to GS 31 total rainfall was 28% higher in 2013/14 than in 2012/13. In contrast in the following period from GS 31 to GS 75 2013/14 was wetter than 2012/13. Overall mean temperature from GS 15 to GS 75 showed conditions were warmer in 2013/14 than 2012/13. Relative humidity was higher in 2012/13 experiment from sowing until GS 31 than in 2013/14. In the following period from GS 31 to GS 39 relative humidity was 7.5% higher in 2013/14 than 2012/13. However, from GS 39 to GS 75 relative humidity was higher in 2012/13 than 2013/14.

Table 5.30 Mean temperature, total rainfall and relative humidity from sowing to GS 75 in 2012/13 and 2013/14 field experiments.

		Sowing to GS 15	GS 15 to GS 31	GS 31 to GS 39	GS 39 to GS 75
Mean temperature (^{o}C)	2012/13	8.0	4.0	6.25	12.8
	2013/14	7.2	6.5	10.5	14.6
Total rainfall (mm)	2012/13	142.0	164.6	87.4	88.6
	2013/14	63.6	211.4	69.4	76.0
Relative humidity (%)	2012/13	88.2	87.4	75.5	77.3
	2013/14	86.2	83.7	81.2	74.9

5.4 DISCUSSION

5.4.1 Plant emergence, root rot and stem base diseases and their pathogens

This is the first study to show *R. cerealis* can significantly reduce the emergence of wheat in UK conditions. In contrast, *R. solani* AG 2-1 and AG 5 only caused marginal reductions in emergence suggesting these species are not major casual organisms of pre or post-emergence damping off in wheat. *Rhizoctonia cerealis* has been previously reported to cause pre- and post-emergence damping-off (Parry, 1990; Hamada *et al.*, 2011a). However, *R. cerealis* is generally not associated with establishment losses. In 2012/13 emergence from untreated seed in the non-inoculated plots was low indicating the presence of other seed or soil borne pathogens. *Microdochium* spp. are seed or soil borne pathogen capable of causing considerable establishment losses, (Millar and Colhoun, 1969; Humpreys *et al.*, 1995). The presence of these species was confirmed in stems at GS 13 with real-time PCR assays. Both *M. nivale* and *M. majus* were found at higher DNA concentrations in stems in 2012/13 than 2013/14. This suggests these species were responsible for the low emergence from untreated seed in 2012/13. In addition, the 2012/13 experiment was sown deeper than the optimum depth of about 4 cm (AHDB, 2015) which will have exacerbating the effect of *Microdochium* spp. on plant emergence.

Throughout the period of the study there was no significant difference in root rot disease index between the inoculated and non-inoculated plots. Previous studies have shown contrasting results on the pathogenicity of isolates of AG 2-1 and AG 5 to wheat roots (Roberts and Sivasithamparam, 1986; Ogoshi *et al.*, 1990; Rush *et al.*, 1994; Khangura *et al.*, 1999). The contrasting reports are likely due to differences in the aggressiveness of individual isolates of AG 2-1 and AG 5 to wheat. The results from this study suggest the AG 2-1 and AG 5 isolates were only weakly pathogenic to wheat roots. Therefore, future studies should investigate the aggressiveness of a range of naturally occurring isolates. Although real time PCR assays detected AG 2-1, AG 5 and *R. cerealis* DNA in roots indicating these pathogens were able to colonise wheat roots. That said, no association found between *Rhizoctonia* spp. DNA in roots and root rot symptoms. This suggests the presence of other root rot causing pathogens. Root rot is

generally not caused by a single pathogen but by a complex of species in the rhizosphere that cause disease with very similar symptoms (Harris and Moen, 1985; Raaijmakers *et al.,* 2009).

Stem base diseases at early growth stages of the host are difficult to distinguish (Turner *et al.,* 1999; Turner *et al.,* 2001). Therefore, plants were assessed at GS 15 for general stem browning. The highest stem browning consistently occurred in *R. cerealis* inoculated plots in both experiments. In addition, *R. cerealis* DNA was also quantified at higher concentrations than AG 2-1 and AG 5. This suggests wheat was more susceptible to infection by *R. cerealis* than AG 2-1 or AG 5. Indeed the effect of *R. cerealis* on plants at GS 15 was to significantly reduce plant height resulting in the appearance of stunted plants. Parry, (1990) suggested that stem browning in the autumn is often the result of sharp eyespot infections. However, growers and agronomists often associate stem browning at the early growth stages of wheat with *Microdochium* spp. This study showed *R. cerealis* is capable of causing infections early in the season that can significantly affect plant growth. *Rhizoctonia solani* AG 5 also caused considerable stem browning in 2012/13 when pathogen DNA in soil of plots sown with untreated seed was >1400 fold higher than in 2013/14. In a controlled environment study similar symptoms by this pathogen have previously been reported on three week old wheat seedlings (Woodhall *et al.,* 2012b). Differences in environmental conditions may have affected pathogen accumulation in soil and disease by AG5. For example, rainfall and atmospheric temperatures were higher from sowing to GS 15 in 2012/13 compared to the same period in 2013/14 (Table 5.30). The acute stem browning on seedlings from untreated seed in the non-inoculated control plots in 2012/13 suggested the presence of other soil-borne pathogens. Indeed, DNA of the *Microdochium* spp. was quantified at relatively high amounts in stems at GS 13 in 2012/13 compared to 2013/14 and therefore, these species are likely to be responsible for some stem browning in the non-inoculated control. Since DNA of *Rhizoctonia* rather than *Microdochium* accounted for 60% of the variation in stem browning at GS 15 we can be confident that *Rhizoctonia* spp. were responsible for the majority of symptoms in inoculated plots at GS 15.

Overall the highest sharp eyespot index occurred at GS 31. This is earlier than expected in naturally infected fields where symptoms have been recorded mostly

after stem extension (Polley and Turner, 1995; Lemańczyk and Kwaśna, 2013). Early disease occurrence and severity is likely due to the inoculation methods that delivered high amounts of inoculum directly next to the sown seed thus allowing the pathogen to colonise the developing seedling prior to tillering (GS 31). As sharp eyespot symptoms were also present in the non-inoculated, AG 2-1 and AG 5 inoculated plots it is likely that misdiagnosis of symptoms occurred. This is supported by the results of soil samples taken from each site for DNA extractions prior to drilling that failed to reveal the presence of AG 2-1, AG 5 or *R. cerealis*.

BFR index was significantly lower in *R. cerealis* inoculated plots than AG 2-1, AG 5 or the non-inoculated control. This was also shown in the weak but significant negative correlations between sharp eyespot and BFR index. This would suggest interspecific competition between *R. cerealis* and BFR causing species. Positive interactions between *M. majus* and both *O. acuformis* and *R. cerealis* have previously been suggested (Turner *et al.,* 1999). Bateman (1993) also reported that the *Oculimacula* spp. were co-isolated along with *M. nivale* at a higher frequency than just by chance. However, *Microdochium* spp. DNA in stems at GS 15 in *R. cerealis* plots was not significantly lower than in AG 2-1 and AG 5 plots although this could be too early to detect differences. It may also indicate that *R. cerealis* had a competitive advantage as a stem coloniser over other BFR causing species. BFR can be caused by a range of *Fusarium* spp. especially *F. culmorum* and *F. avenaceum* (Pettitt *et al.* 2003). Further PCR assays targeting *Fusarium* spp. could be used to investigate any potential competitive advantage for *R. cerealis* over BFR causing species.

5.4.2 Effects of seed treatment on root rot and stem base diseases and their pathogens diseases and pathogens

Fludioxonil and fludioxonil + sedaxane increased plant emergence by 47.9 and 50.2% respectively in 2012/13. Therefore, under high disease pressure caused by a mixture of *Rhizoctonia* spp. and *Microdochium* spp, there was a significant response to both seed treatments. Fludioxonil (Gylnn *et al.,* 2007; Gylnn *et al.,* 2008) and sedaxane (Zeun *et al.,* 2013) have been previously shown to effectively control *Microdochium* spp. *in vitro* and in field. Furthermore in this study

sedaxane as part of a mixture with fludioxonil, under high disease pressure from *R. cerealis* significantly increased emergence by 43% and 54% compared to fludioxonil alone and the untreated respectively. This demonstrated an additional effect of sedaxane in controlling damping off caused by *R. cerealis*. This is in agreement with studies, investigating the effective concentration at which 50% (EC50) of mycelial growth of *Rhizoctonia* spp. was inhibited, that demonstrated the high activity of sedaxane against *R. cerealis* (Zeun *et al.,* 2013; Goll *et al.,* 2014).

The seedling stage of development is when roots are most susceptible to attack from soil borne pathogens and therefore most in need of protection. In this study root rot at GS 15 was reduced by seed treatment compared to the untreated. Although not significant (SED at 5%) there was an additional effect of sedaxane as part of mixture with fludioxonil reducing root rot by 6% compared to fludioxonil alone. Previous reports in greenhouse studies have shown sedaxane to significantly reduce rhizoctonia root rot in maize, corn and cotton (Zeun *et al.,* 2013). This study has shown sedaxane was able to reduce root rot on winter wheat in field conditions.

The effect of seed treatment at later growth stages was less consistent especially in AG 5 inoculated plots. This is not surprising as the main period of activity of a seed treatment is generally considered to last 4-6 weeks after sowing. However, visual assessments of root rot symptoms are not associated with individual species but a complex of organisms (Cook *et al.,* 2002). This disadvantage can be overcome by quantifying pathogen DNA in roots. Results showed no consistent effect of seed treatment on DNA of AG 2-1, AG 5 or *R. cerealis* in roots. Therefore, seed treatments consistently reduced root rot symptoms but not AG 2-1, AG 5 or *R. cerealis* DNA.

Sedaxane + fludioxonil treatments consistently reduced stem browning in both seasons. Fludioxonil alone was less consistent since its effect was only significant in the 2012/13 experiment. This suggests an additional beneficial effect of sedaxane in controlling stem diseases caused by *Rhizoctonia* spp. and *Microdochium* spp. at early crop growth stages. It is also possible that a synergistic effect between sedaxane and fludioxonil in controlling *Microdochium*

spp. exists. Previous field trials established in England, Germany and Switzerland showed improved control of disease caused by both *Microdochium* spp. when applied in mixture to seed (Zeun *et al.,* 2013). However, lower disease and greater consistency were achieved with the three way mixture of sedaxane + fludioxonil + difenoconazole (Zeun *et al.,* 2013). There was no significant effect of seed treatment at 5% SED on pathogen DNA although *Rhizoctonia* DNA concentrations were lowest in sedaxane + fludioxonil treated plots. Quantification of *Microdochium* spp. DNA showed fludioxonil was more consistent in controlling *M. nivale.* While no *M. majus* DNA was detected in sedaxane + fludioxonil treatments in both seasons suggesting sedaxane was effective in controlling this species. These two active ingredients with different modes of action can be used against *Microdochium* spp. in combination as a resistance management strategy (Zeun *et al.,* 2013). Resistance of *Microdochium* spp. has been shown in France towards strobilurin fungicides (Walker *et al.,* 2009). Although no current resistance to fludioxonil or sedaxane is known therefore this combination would be effective to avoid resistance developing in *Microdochium* spp.

The lack of effect of seed treatment on sharp eyespot after GS 31, 39 or 75 suggests that sharp eyespot control may require additional foliar fungicides that should be applied at the beginning of internode elongation (GS 30–31) (Nicholson *et al.,* 2002). However, sedaxane + fludioxonil treatments had significantly lower BFR index at GS 31 and GS 39 in both experiments compared to the untreated. This shows that sedaxane + fludioxonil treatments provided continued protection from BFR causing species up to GS 39. *Microdochium* spp. are considered the principle pathogens causing BFR (Turner *et al.,* 2002). However, other *Fusarium* spp. such as *F. culmorum* and *F. avenaceum* can also cause stem browning especially at later growth stages (GS 73-75) (Pettitt *et al.,* 2003).

5.4.3 Spectral reflectance measurements

Infections by *Rhizoctonia* spp. cause disease on the roots and stems of plants and this can reduce the absorption and translocation of water and nutrients. This places the plant under stress and impacts on yield as, for example, water is a limiting factor in photosynthesis (Tezara *et al.,* 1999). These effects can be detected in the visible and/or near-infrared regions of the electromagnetic spectrum (Naidu *et al.,* 2009). Therefore, spectral reflectance measurements (SRM) can be used to detect differences in the health status of healthy and diseased plants (Sankaran *et al.,* 2010). This study used SRM to investigate if effects of soil borne pathogens can be detected in the canopy. Overall spectral reflectance indices in this study did not show any clear differences between inoculated and non-inoculated from untreated seed; especially at the earliest growth stage (GS 39) when such methods could be used to inform crop protection decisions. However, G-NDVI and NWI-1 at GS 39 were significant in the MRL analysis for grain yield. These indices are linked to photosynthetic capacity and plant water and nutrient status. Previous studies have shown SRM can detect plant diseases. Inoculated field trials in China showed that photochemical reflectance index (PRI) showed a significant negative relationship (R^2 = 0.91) with yellow rust (Huang *et al.,* 2007). Graeff *et al.* (2006) in a glasshouse study was able to detect and distinguish between plants with powdery mildew and take-all infections using leaf reflectance measurements. They suggested differences may be due to changes in pigments or nutrient status of plants. The lack of differences in this study suggests these species only had a marginal effect on SRM's or that this method was not appropriate to detect the effect of these pathogens. However, in this study seed treatments did have an effect on spectral reflectance indices. Overall PRI was significantly lower by 3.5% in sedaxane + fludioxonil than the untreated at GS 39. However, at GS 85 the reverse was shown with PRI 9.3% higher in sedaxane + fludioxonil treatments. PRI provides an index of photosynthetic radiation use efficiency (PhRUE) (Gamon *et al.* 1997). This would suggest PhRUE was reduced in sedaxane + fludioxonil treatments at GS 39 but enhanced at the end of the season GS 85.

5.4.4 Yield components

Reduction in yield components was most associated with effects of *R. cerealis.* The two main yield components affected were plant height at GS 13 and grain yield. Therefore, *R. cerealis* impacted on crop growth throughout the plants lifecycle. Overall grain yield in *R. cerealis* inoculated plots was reduced by 8.5% compared to the untreated. The yield reductions caused by *R. cerealis* in this study are similar to those reported in previous studies. Clarkson and Cook (1983) in the UK found average yield losses over five seasons were 5% for moderate infections. More recently Lemańczyk and Kwaśna (2013) estimated maximum yield losses in Poland were 8-10% from natural infested commercial wheat fields. Good negative correlations were found between yield and eyespot index at GS 31 which accounted for 40% of the variation in yield. However, eyespot index was not significant in the yield MLR model.

The most consistent effect of seed treatment was on plant height at GS 13 by negating the effects of soil-borne pathogens on this trait. However, sedaxane + fludioxonil were more effective than fludioxonil in 2013/14 when plant height was decreased to a greater extent in *R. cerealis* plots. Seed treatments did not significantly increase grain yield. Previous field trials using the same method to inoculate the soil with *R. cerealis* as this study have shown sedaxane to increase wheat yields (Zeun *et al.,* 2013). The results showed that sedaxane solo or in combination with fludioxonil and/or difenoconazole increased yields of wheat yields compared to untreated seed in *R. cerealis* inoculated plots (Zeun *et al.,* 2013). The results from this study would suggest that to prevent yield losses from *R. cerealis* infections further control measures (e.g. foliar fungicides) are maybe required.

5.5 CONCLUSIONS

The results within this chapter enable the following conclusions to made:

i. *Rhizoctonia cerealis* is capable of causing significant reductions in emergence and high early disease severity resulting in stunted growth of winter wheat seedlings in UK conditions.

ii. *Rhizoctonia cerealis and R. solani* AG 5 were the main Rhizoctonia species associated with significant stem browning.

iii. Sharp eyespot symptoms were always associated with *R. cerealis* throughout the period of study.

iv. BFR was significantly reduced in *R. cerealis* inoculated plots. This suggests that *R. cerealis* was able to outcompete BFR causing species. However, this may only occur if *R. cerealis* infects the stem earlier than BFR causing species.

v. *Rhizoctonia cerealis* significantly reduced grain yield by 8.5% compared to the non-inoculated control. *Rhizoctonia solani* AG 2-1 caused negligible yield losses of 2.5% and AG 5 did not reduce yield of winter wheat.

vi. Fludioxonil and sedaxane + fludioxonil significantly increased emergence of winter wheat in 2012/13 when there was high disease pressure from *Microdochium* spp.

vii. Sedaxane + fludioxonil were active against *R. cerealis* significantly increasing emergence of winter wheat by 54% compared to the untreated in 2013/14.

viii. Stem browning severity caused by *Rhizoctonia* spp. and *Microdochium* spp. was consistently reduced by sedaxane + fludioxonil compared to the untreated in both experiments.

ix. Overall BFR severity was significantly lower in sedaxane + fludioxonil treatments at GS 31 and GS 39 than in the untreated.

x. Fludioxonil and sedaxane + fludioxonil significantly increased plant height at GS 15 when there was high disease pressure from *Rhizoctonia* spp. and *Microdochium* spp.

Chapter 6

Characterisation of *Rhizoctonia* isolates from English wheat crops

6.1 INTRODUCTION

Several methods have been used to characterise isolates of *Rhizoctonia* spp. These include but not exclusively morphology, DNA analysis, fungicide sensitivity, pathogenicity, DNA analysis, hyphal fusion compatibility and optimum temperature for growth. These methods have enabled the identification of new AGs and sub-groups within AGs. The continued phenotypic and genotypic characterisation of *Rhizoctonia* spp. is vital to classify new isolates or sub-groups, determine differences between isolates, monitor population changes and to identify shifts in fungicide sensitivity. This chapter uses three methods to characterise isolates of *Rhizoctonia* spp. isolated and used in this study.

The aim of this work was to characterise a range of multinucleate and binucleate *Rhizoctonia* spp. using ITS sequencing (section 6.2), isolate pathogenicity towards wheat seedlings (section 6.3) and *in vitro* fungicide sensitivity (section 6.4).

6.2 rDNA-ITS sequencing of *Rhizoctonia* spp.

6.2.1 Introduction

Sequences obtained from the internal transcribed spacer (ITS) have been used in DNA barcoding to identify species, sub-species and to investigate evolutionary relationships among species (Schoch *et al.,* 2012). DNA barcoding is a taxonomic method that uses short, homogenous gene regions to distinguish closely related species (Herbert and Gregory, 2005). The most commonly used barcode in fungi is the internal transcribed spacer (ITS). The ITS region is part of the ribosomal DNA (rDNA) that shows a wide degree of variation in fungi and is commonly used as the standard barcode sequence to identify species and sub-groups (Schoch *et al.,* 2012). Sharon *et al.* (2006 & 2008) reviewed a number of characterisation techniques used for the identification and classification of multinucleate and binucleate *Rhizoctonia* spp. These included biochemical (e.g. isozyme analysis), molecule (e.g. sequencing) and the classical anastomosis groupings based on hyphal fusion. The reviews found the most suitable approach to classify *Rhizoctonia* spp. was the sequencing of the rDNA-ITS region.

An investigation into the genetic diversity of *R. solani* isolates found the rDNA-ITS region to be highly heterogeneous (Kuninaga *et al.* 1997). Heterogeneity of the ITS region has enabled the confirmation of sub-groups within some *R. solani* AGs. For example, to date three subgroups (IA, IB and IC) have been identified for AG 1. Sequencing the rDNA-ITS region of the binucleate species *R. cerealis* has also identified three sub-groups (I, II, III) (Hayakawa *et al.,* 2006; Sharon *et al.,* 2008). The three subgroups of *R. cerealis* have been shown to differ in both virulence and in diseases caused towards turfgrass; for example, AG D-I causes rhizoctonia-patch and winter-patch disease while AG D-II causes elephant footprint disease (Toda *et al.*, 1999; Hayakawa *et al.*, 2006). It has also been suggested that the principle sub-group causing sharp eyespot in wheat is AG D-I (Li *et al.*, 2014). However, the occurrence of *R. cerealis* sub-groups in UK wheat crops is unknown.

The objectives of this work were to:

i) Investigate the sequence heterogeneity of the rDNA-ITS region of *Rhizoctonia* isolates cultured from wheat plants in England.

ii) Identify the predominant sub-group of *R. cerealis* occurring in English wheat crops.

The specific null hypotheses tested within this study were:

i) Sequence comparison of the rDNA-ITS region of all *Rhizoctonia* isolates cultured from wheat plants in England will be the same.

ii) There will be no predominant sub-group of *R. cerealis* in English wheat crops.

6.2.2 Materials and methods

6.2.3 ITS sequencing of *Rhizoctonia* spp.

6.2.4 Origin and collection of isolates

This experiment investigated the sequence heterogeneity of the ITS region of *Rhizoctonia* spp. isolated from English wheat crops. Wheat plants were sampled at GS 21-31 in 2012 and GS 65 in 2014 from distinct geographical regions across England. Plants sampled at GS 21-31 in 2012 were collected from the 52 fields described in chapter 3 following the method set out in section 3.2.1. Isolations were taken from roots and stems of both symptomatic and asymptomatic plants. At GS 65-75 an additional seven winter wheat fields were sampled, plants in these fields were assessed *in situ* for sharp eyespot and only those with symptoms removed. This was to maximise the number of isolates collected.

Isolations were performed on root and stem sections (5-10 mm). Sections were surface sterilised in 0.5% sodium hypochlorite (NaOCI) for one minute then rinsed three times in sterile distilled water for no longer than 30 s. Sections were placed on filter paper (grade 1) in an open Petri dish and dried for 30-60 minutes in a flow cabinet to prevent bacteria growth on agar plates. Six pieces of root or stem were then transferred to tap water agar (TWA) plates amended with 0.1 g l^{-1} of streptomycin and penicillin. After 48-72 h incubation at 20^{o}C hyphal tips were examined under a dissection microscope for colonies of *Rhizoctonia* spp. Colonies were then transferred to PDA plates and stored at 20^{o}C for no longer than three weeks until DNA extractions were performed.

6.2.5 DNA extraction

DNA from isolates was extracted using the method presented in section 2.2.3.1 of chapter 2.

6.2.6 PCR

BIO-X-Act™ Shortmix was used for all PCR (comprising of half the 25 µl mastermix). A Biorad C1000 was used with the cycling conditions consisting of an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 58°C for 45 s and 72°C for 60 s, and a final extension step at 72°C for 5 m. Forward and reverse primers consisted of 400 nM of the final volume. 1 µl of template DNA was used and nuclease free water was used to make up to 25 µl in the reaction. Products were run on 1.5% agarose gels stained with 10 µL of GelRed® per 100 ml. Gels were ran at 120V for 50 minutes. Gel images were taken with a UVP BIODOCT-IT ™ System at 0.6 seconds of light exposure. Primers used throughout were ITS1 and ITS4 (White *et al*. 1990) for standard DNA barcoding.

6.2.7 DNA sequencing

To purify the PCR products a QIAquick PCR Purification Kit was used. Samples were sent to MWG Biotech for DNA sequencing according to their instructions. DNA was sequenced in both directions and the resulting sequences were aligned and subsequent sequence analysis was undertaken using MEGA v6 (Tamara *et al*., 2013). Phylogenetic analysis was used as described in Woodhall *et al*., (2007) to show phylogenetic relatedness between *R. cerealis* isolates. Briefly, the construction of distance matrix values was performed using the neighbour joining method. Distances in the rDNA ITS region was calculated using Kimura's two parameter model, omitting sites and gaps. Bootstrap analysis was performed with 1000 re-samples of data. The rDNA ITS sequence of the binucleate AG-E was used as an out-group.

6.2.8 Results

6.2.9 Anastomosis groups

In total 161 isolates with *Rhizoctonia* like morphology were cultured from wheat samples during 2012 (GS 21-31) and 2014 (GS 65-75), of which 160 isolates were identified as *R. cerealis* (Table 6.1). There was also one potential new *Rhizoctonia* sp. (1739) isolated at GS 21-31 from stem material. This isolate had distinct morphological features of white fluffy hyphae close to the surface of the PDA plate. At GS 21-31 (2012) 52 wheat fields were sampled and 27 *Rhizoctonia* isolates were obtained from 11 fields. *Rhizoctonia cerealis* DNA was present in root and stem samples in 10 of the 11 fields where isolates were cultured (Table 6.1). In 2014 a further seven wheat fields were sampled at GS 65-75 with 133 isolates of *R. cerealis* cultured from stems with visible sharp eyespot lesions. No PCR assays were performed and no other *Rhizoctonia* spp. were cultured from these seven fields. In total three isolates were cultured from roots and the remaining from stems of wheat.

6.2.10 Analysis of rDNA ITS sequence diversity in *Rhizoctonia* spp.

The 160 UK isolates of *R. cerealis* were sequenced and aligned in Mega 6. Based on the type of sequence polymorphism at each nucleotide position isolates were assigned to an ITS type. In total 24 unique ITS types were present in English isolates (Figure 6.1). ITS type 'RC2' predominated with 25 isolates. This was followed by ITS type RC5 (18 isolates), RC9 (17 isolates), RC18 (15 isolates) RC21 (14 isolates) and RC15 (12 isolates). All other ITS types were found at in <10 isolates with six ITS types represented by just one isolate.

Table 6.2 shows the ITS types of *R. cerealis* isolates present in each field and county from which they were cultured. Multiple types were present in the same field/county but there was no relationship between field/county and the ITS type present. For example, ITS type RC2 was present in six fields across four counties and RC5 was present eight fields across six counties.

Based on the ITS regions a neighbour joining tree was constructed using sequences of the *R. cerealis* isolates obtained in this study with reference sequences from Genbank (Figure 6.2). Three clearly visible groups can be seen

which correspond to previously proposed AG D (I, II, III) groups for the *R. cerealis*. Despite evidence of intra-species variation all UK isolates were assigned to group D-I.

		Nucleotide number of polymorphism															
ITS type	No. of isolates	69	80	85	104	118	151	155	161	204	220	237	246	280	426	457	459
RC1	7	A	G	T	T	R	G	T	G	A	A	T	A	C	A	A	T
RC2	25	A	R	T	F	A	R	T	F	R	A	T	A	C	A	A	Y
RC3	2	R	G	T	F	A	G	T	G	A	A	Y	A	C	A	A	T
RC4	4	A	R	T	F	R	G	Y	G	R	R	T	A	Y	A	A	T
RC5	18	A	R	T	F	A	G	T	G	R	R	T	A	C	A	A	T
RC6	1	A	G	T	T	A	G	T	G	A	A	Y	A	Y	A	A	T
RC7	4	A	R	T	F	A	G	T	G	R	A	T	A	C	A	R	T
RC8	1	A	G	T	T	R	G	T	G	A	A	Y	A	Y	A	A	T
RC9	17	A	R	T	F	R	R	T	G	R	A	T	A	Y	A	A	T
RC10	2	A	R	T	F	A	R	T	G	R	A	T	A	C	A	A	T
RC11	1	A	G	T	T	A	G	T	G	A	A	Y	A	C	R	A	T
RC12	4	A	R	T	F	A	R	T	G	R	A	T	A	C	A	R	T
RC13	3	A	G	T	T	A	G	T	G	A	A	Y	A	C	A	A	T
RC14	6	A	R	T	F	A	G	Y	G	R	R	Y	A	C	A	A	T
RC15	12	A	R	T	F	A	R	T	F	R	A	Y	A	C	A	A	Y
RC16	1	A	R	T	F	A	G	T	G	R	R	Y	A	C	A	A	T
RC17	9	A	R	Y	F	R	G	T	G	R	R	T	A	Y	A	R	T
RC18	15	A	R	Y	F	A	G	T	G	R	R	T	A	C	A	R	T
RC19	1	A	R	Y	F	A	G	T	G	R	R	Y	A	C	A	R	T
RC20	6	R	G	T	F	R	G	T	G	A	A	Y	A	Y	A	A	T
RC21	14	A	R	T	F	R	R	T	F	R	A	T	A	Y	A	A	Y
RC22	4	A	R	T	F	R	G	T	G	R	A	T	A	Y	A	R	T
RC23	2	R	G	T	F	A	G	T	G	A	A	T	R	C	A	A	T
RC24	1	A	R	T	F	A	R	T	G	R	A	Y	A	C	A	A	T

Figure 6.1. Number of rDNA ITS sequence variants in 160 UK isolates of *R. cerealis* and corresponding nucleotide positions of their polymorphism

Table 6.1 Distribution (field and county) of the 160 *R. cerealis* isolates cultured from English wheat crops along with ITS type sequenced and presence (✓) or absence (×) of *R. cerealis* DNA

						R. cerealis* DNA (GS21-31)		
County	**Field**	**Cultured from**	**GS**	**No. isolates**	**ITS type**	**Soil**	**Roots**	**Stems**
Lancashire	2nd WW	Stems	21-31	2	10	✓	✓	✓
N. Yorkshire	1st WW	Roots (1 isolate) Stems	21-31	2	2,5	×	✓	✓
N. Yorkshire	2nd WW	Stems	21-31	2	3	×	×	✓
Staffordshire	R. Brown	Stem	21-31	2	2	✓	✓	✓
Suffolk	4 winds	Roots (1 isolate) Stems	21-31	5	4,5	×	✓	✓
Suffolk	Pill box	Stems	21-31	2	2	✓	✓	✓
Surrey	Barton's	Stems	21-31	4	6,7,9	✓	✓	✓
Surrey	Green park	Roots (1 isolates) Stems	21-31	2	5	✓	✓	✓
W. Yorkshire	1st WW	Stems	21-31	1	1	✓	✓	×
W. Yorkshire	2nd WW	Stems	21-31	2	9	✓	✓	✓
Wiltshire	Venezuela	Stems	21-31	1	8	×	✓	✓
Cornwall	Sawmills	Stems	65-75	17	1,5,7,9,13,14,15,16,17			
N. Yorkshire	50 acre	Stems	65-75	10	2, 5, 12, 14			
Nottinghamshire	S24	Stems	65-75	52	1, 2, 5, 7, 9, 15, 20, 21, 22			
Nottinghamshire	Field 1	Stems	65-75	17	2, 15, 17, 23			
Shropshire	Field 1	Stems	65-75	26	4, 5, 12, 15, 18, 24			
Shropshire	Field 2	Stems	65-75	11	5, 7, 11, 14, 18, 20, 21			
Shropshire	Field 3	Stems	65-75	2	5,17			

* Presence or absence of pathogen DNA was only established at GS 21-31 using the data presented in chapter 3

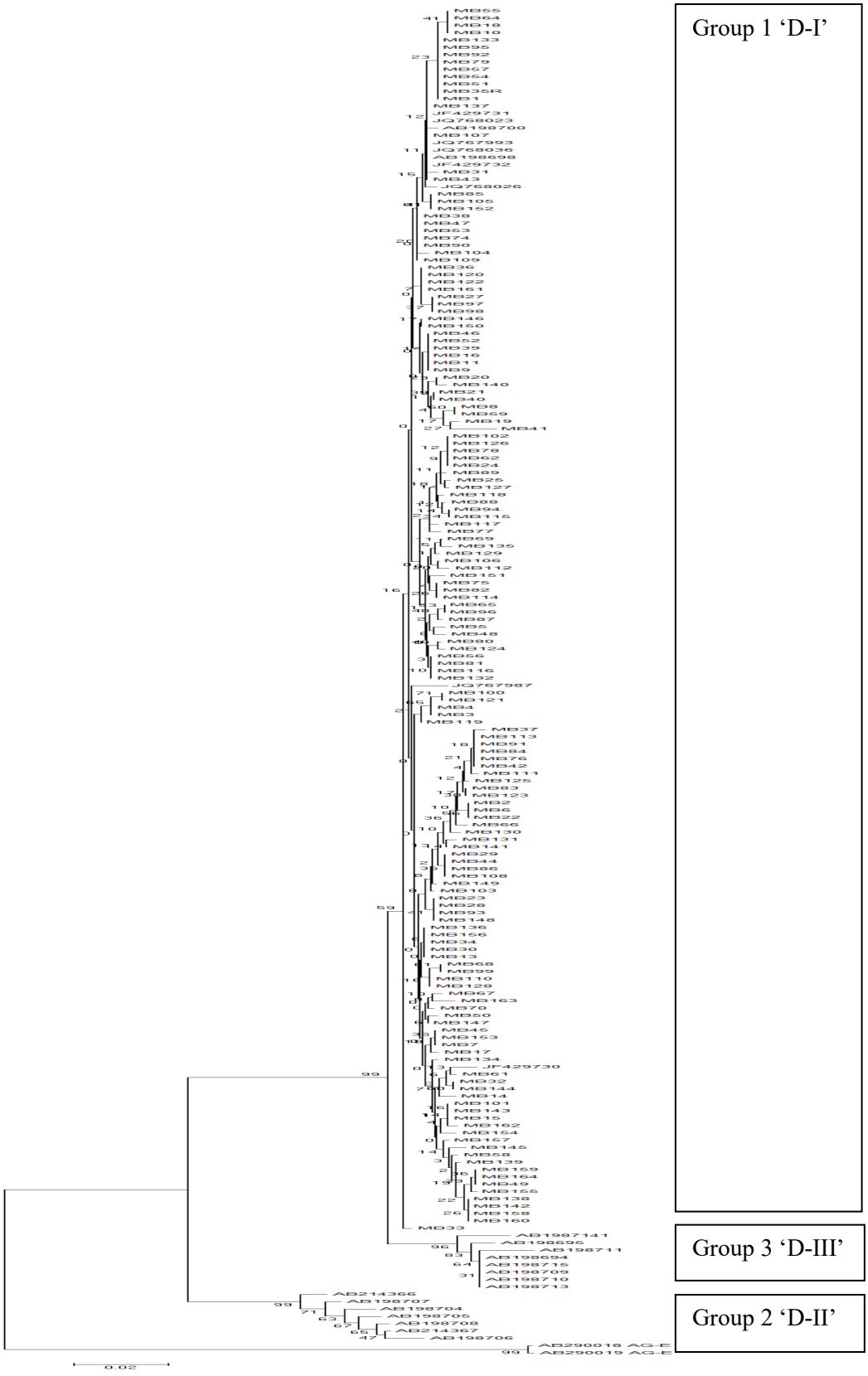


Figure 6.2. Phylogenetic tree based on the rDNA-ITS sequences of UK *R. cerealis* (AG-D) isolates (labelled MB1 to 160) with reference sequences from GenBank. The tree was built using the neighbour joining method. The numbers at branches were the percentage of trees containing the corresponding clade based on 1,000 bootstrap replications. AG-E was used as an outgroup

6.2.11 Discussion

6.2.12 Anastomosis groups

Isolations performed on wheat samples collected at GS 21-31 and GS 65-75 yielded 160 *R. cerealis* isolates. The large number of *R. cerealis* isolates collected show how easily this species is cultured from plant material. Interestingly, three isolates were obtained from wheat roots. This is the first study to isolate *R. cerealis* from the roots of wheat. However, no AG's of *R. solani* were obtained from wheat roots or stems in this study. Isolating *R. solani* from the roots and stems of wheat is difficult (Pers. Comm. Woodhall, J.). The preferred option to obtain field isolates is via soil isolations; with the toothpick method considered the most successful (Kumar *et al.*, 1999; Paulitz and Schroeder, 2005). One potential new *Rhizoctonia* spp. was identified but further analysis is required to classify this isolate.

6.2.13 rDNA ITS sequencing

In total 160 *R. cerealis* isolates were collected and DNA extracted and the ITS region sequenced. In this study we found 24 unique ITS regions within 160 *R. cerealis* isolates are present in English wheat crops. Isolates collected from the same field showed considerable variation within the ITS region; for example one field in Cornwall yielded 17 isolates which had nine unique sequences. Heterogeneity is also known to occur in the ITS region within the same isolate, with some isolates showing several unique sequences per isolate (Li *et al.*, 2014). However, isolates in this study were not analysed for multiple unique ITS sequences. Isolates of *R. cerealis* have been divided into three sub-groups, AG D-I, II and III that have shown to differ in their pathogenicity towards some *Poaceae* species (Hayakawa *et al.*, 2006; Sharon *et al.*, 2008). The ITS sequence analysis revealed all 160 isolates belonged to AG D-I. This is the first study to show that the predominant pathogen causing sharp eyespot in England is AG D-I. A similar result was also found in China were sharp eyespot is a serious pathogen of wheat. Li *et al.* (2014) sequenced the ITS region of 15 isolates collected from wheat plants with sharp eyespot symptoms and showed that all 15 isolates belonged to AG D-I. This suggests the main sharp eyespot causing species is AG D-I. The sub-groups, AG D-II and AG D-III are known to cause disease on turfgrass (Hayakawa *et al.*, 2006) but have not been found on wheat. This

suggests that while all three sub-groups can cause disease in a range of *Poaceae* spp, but that wheat seems to be specifically infected by AG D-I (Li *et al.*, 2014). It is likely that AG D-II and AG D-III are present in the UK but in other production systems such as turf-grass and horticulture.

6.3. Aggressiveness of *Rhizoctonia* spp. to wheat

6.3.1 Introduction

Rhizoctonia spp. cause damping-off, hypocotyl and coleoptile rots of seedlings and, as plants develop, root and crown rot in a range of crops. *Rhizoctonia solani* is a known pathogen of wheat with AG 8 the most commonly associated AG. However, other AG's of *R. solani* are also known to infect wheat, yet reports of the various AG's causing disease are contrasting. *R. solani* AG 2-1 has been found to be pathogenic (Roberts and Sivasithamparam, 1986) and non-pathogenic to wheat seedlings (Khangura *et al.*, 1999). Tewoldmedhin *et al.* (2006) found AG 2-2 to cause significant damping-off. However, Rush *et al.* (1994) found there was no effect on the emergence of wheat from AG 2-2. Similar contrasting reports have also been reported for AG 4, 5 and 11 (Demirci, 1998; Ogoshi *et al.*, 1990; Tewoldmedhin *et al*., 2006; Woodhall *et al.*, 2012b).

Rhizoctonia cerealis is generally associated with causing sharp eyespot and to lesser extent pre- and post-emergence damping-off (Pitt, 1966; Hamada *et al.* 2011a). There have been reports of the increasing incidence of sharp eyespot in various countries including Poland (Lemańczyk 2010), the UK (Colbach *et al.* 1997b) and China (Chen *et al.* 2010). Additionally, it has been proposed that the severity of sharp eyespot has increased in the last two decades due to climate change (Hamada *et al.*, 2011a). However, other factors such as earlier sowing, growing of susceptible cultivars and movement of aggressive isolates from natural plant communities to the field environment have also been implicated in the changing disease severity (Chakraborty *et al.* 2000; Lemańczyk and Kwaśna, 2013). Although, sharp eyespot is generally not considered a major disease, severe infections can significantly impacting on yields (Hamada *et al.* 2011a). Changes in the severity of disease are likely to be due to the aforementioned factors. However, differences in the aggressiveness of *R. cerealis* isolates have not been previously investigated and could be a factor in the severity of disease.

The objective of this work was to:

i) Investigate the pathogenicity of a range of binucleate and multinucleate *Rhizoctonia* spp. towards winter wheat seedlings.

The specific null hypothesis tested within this study was:

i) Binucleate and multinucleate *Rhizoctonia* spp. will be non-pathogenic to winter wheat seedlings.

6.3.2 Materials and methods

6.3.3 Isolates and experimental design

This experiment investigated the pathogenicity of binucleate and multinucleate *Rhizoctonia* spp. towards winter wheat seedlings. Forty isolates of the 160 *R. cerealis* isolates collected from wheat plants were investigated (section 6.2). The 40 isolates represented at least one of the 24 unique ITS regions identified in sections 6.2. In addition 23 isolates of other *Rhizoctonia* spp. originating from a range of crops and locations were also screened. Appendix 10 lists the details of isolates used in this study.

The pathogenicity experiment was conducted at 18^{o}C with 80% RH and a 16/8 h light/dark period in controlled environment rooms at Fera, Sand Hutton, York. Winter wheat seeds (cv. Santiago) were surface sterilised in 70% ethanol for 30 seconds and then in 10% sodium hypochlorite (NaClO) for 5 mins. The seeds were then rinsed three times in sterile distilled water. Seeds were pre-germinated prior to planting to allow the effect of pathogen on emergence to be accurately quantified. Approximately 30 seeds were placed in a Petri dish with grade 1 filter paper and 5 ml of water and left to imbibe for 24 h. Seeds were discarded from the trial if no visible radicle had emerged, confirming germination, after 24 h. Pots (80 mm height and 40 mm bottom diameter) were filled with John Innes potting compost No.2 to within 2.5 cm of the top. Five pre-germinated seeds per pot were planted equal distance apart. Five plugs (6 mm) were taken from 6-7 day old PDA cultures of the appropriate isolate and placed to the side, but not touching, of the seeds. Pots were then filled with compost to produce a sowing depth of 2.5 cm. Pots were held in a controlled environment room for 10 days and watered when required. The pots were set out in a randomised block design with five replications for each isolate and for the non-inoculated control. Due to the large number of isolates screened four experiments were conducted. In total, 40 *R. cerealis* isolates were screened over two experiments which are referred to as experiment 1 (n = 19 isolates) and experiment 2 (n = 21 isolates) in the text. A further 21 isolates belonging to a range of other *Rhizoctonia* spp. and AGs of *R. solani* were also split over two experiments which are referred to as experiment 3 (n = 11 isolates and experiment 4 (n = 11 isolates) in the text. .

6.3.4 Plant growth measurements and visual disease assessments

After 10 days growth the number of seedlings emerged per pot was recorded and converted to a percentage of seeds planted. Plants were then removed from compost and roots and stems washed to remove compost prior to disease assessments. Refer to section 2.4 for visual disease assessment key for root and stem disease. Plant length was recorded from the basal region of the stem base to the tip of the longest leaf.

6.3.5 Statistical analysis

All data was analysed using Genstat® Version 16th Edition for Windows (VSN International Ltd, UK). Analysis of variance was performed on each of the four experiments with data collected on plant growth measurements and disease assessments. Root and stem disease indexes were angular transformed to normalise residual distribution. Data was then presented in figures for easier interpretation.

6.3.6 Results

In experiments 1 and 2 all *R. cerealis* isolates (*n* = 40) were pathogenic to 10 day old wheat seedlings. Seven isolates in experiment 1 and seven in experiment 2 were able to cause significant reductions in the emergence of pre-germinated wheat seedlings after 10 days growth compared to the controls (Figure 6.3). There were also large differences in the emergence of seedlings within the isolates. Isolates 2029 and 1973 in experiment 1 caused the greatest losses with only 28 and 32% of seedlings emerging, respectively. Figure 6.6 shows visually the pre-emergence damping-off caused by isolate 2029. Root rot symptoms were observed from *R. cerealis* isolates in experiment 1 and 2. However, these symptoms were not significant ($P \geq 0.05$) compared to the control (data not shown). In both experiments all *R. cerealis* isolates caused significant disease on the stems (Figure 6.4). The mean stem disease index (DI) was similar in experiment 1 and 2 at 46 and 45.6% respectively. However, there was large variation in the aggressiveness of *R. cerealis* isolates. In experiment 1 stem DI ranging from 19.8 (isolate 2029) to 73.3% (isolate 2053) and in experiment 2 from 34.4 (isolate 2014) to 64.2% (isolate 2076). Plant height (cm) was also reduced by all but one isolate that occurred in experiment 1 (Figure 6.5). The mean height of inoculated plants was similar in experiment 1 and 2 at 9.6and 10.9 cm compared to 17.5 and 17 cm for the non-inoculated controls, respectively. Figure 6.7 shows the reduced length of plants inoculated with isolate 1486 in experiment 2 compared to the control.

In experiment 3 and 4 the other *Rhizoctonia* spp. were observed to vary in pathogenicity to wheat seedlings. Emergence of wheat seedlings was only reduced by two of the AG 4-II (1938 and 1834) isolates (experiment 4) (Figure 6.8). In experiment 3 *R. solani* AG 8 (1841), isolated from wheat in Australia, caused the greatest root rot indices (48%) compared to the other AGs of *R. solani* and the control (Figure 6.9). *Rhizoctonia solani* AG 8 (1841) and AG 9 (1913) also caused significant root rot. In experiment 4 only AG 4-II (1938) cause significant root rot (21.1%) compared to the control but at much lower indices than that of AG 8 (1841) in experiment 3. Rhizoctonia stem browning was observed from a range of *R. solani* AG's in experiment 3 and 4 (Figure 6.10). In experiment 3 *R. solani* AG 5 (1905 and 1906) and AG 4-II (1306) caused the

greatest stem browning, while in experiment 4 *R. solani* AG 4-II (1834 and 1938), AG 2-1 (1856) and AG 2-2-IIB (1858) caused significant rhizoctonia stem browning compared to the control.. Plant height was significantly reduced by AGs of *R. solani* in experiment 3 and 4 compared to the non-inoculated controls (Figure 6.11). In experiment 3, AG 4-II (1306) and AG 5 (1906) reduced plant height, while *R. solani* AG 4-II (1938 and 1834) and AG 2-1 (1856) also reduced plant height in experiment 4. Also in experiment 4 plants inoculated with AG-I isolate and the potential new *Rhizoctonia* spp. had significantly increased plant height compared to the control.

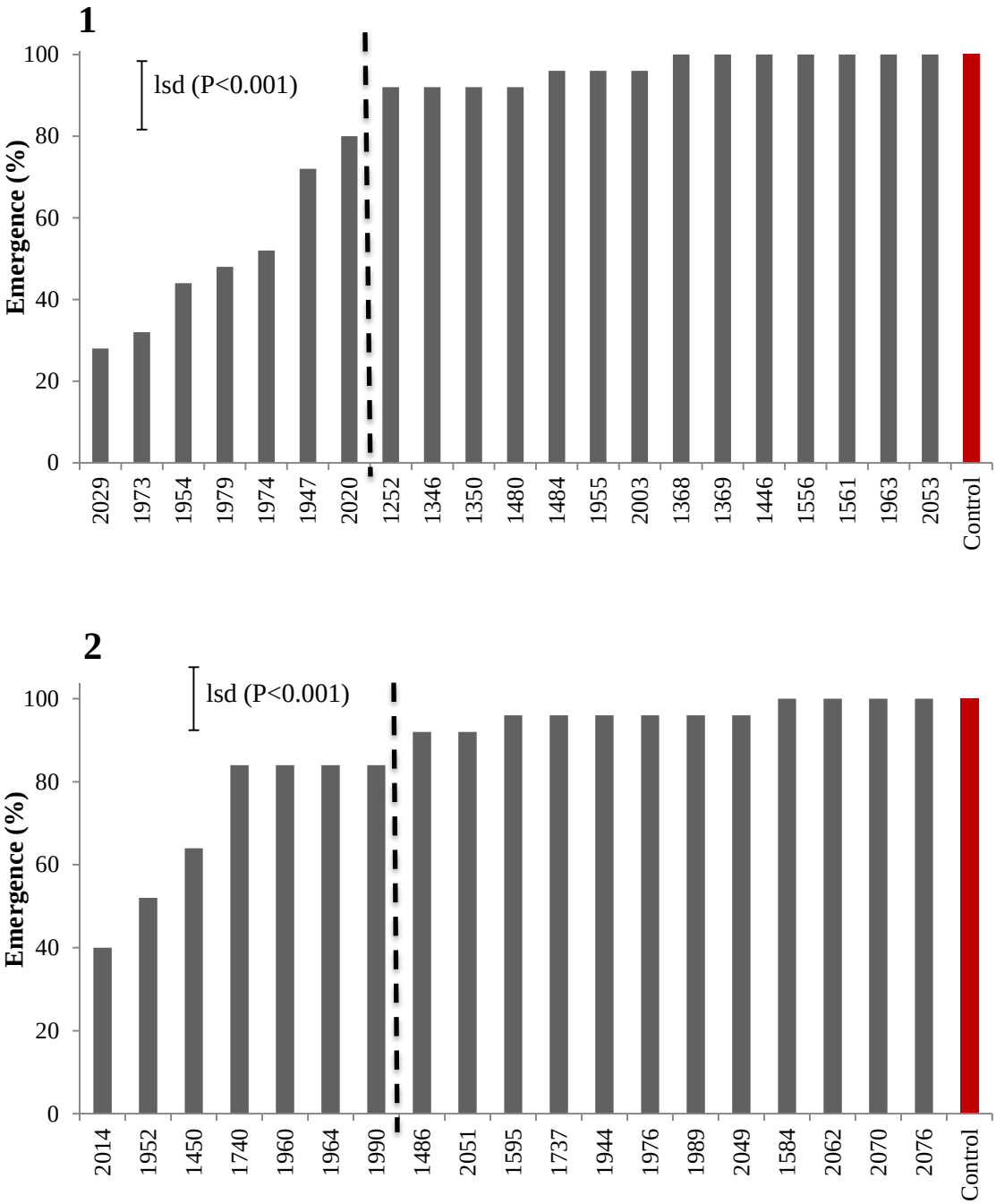


Figure 6.3. Emergence (%) of pre-germinated winter wheat (cv. Santiago) inoculated with *R. cerealis* isolates 10 days post planting conducted over two experiments. 1 = experiment 1 and 2 = experiment 2. Dotted line represents the approximate point where emergence of the inoculated became significantly less ($P < 0.05$) than the emergence for the unon-inoculated control (red line). Bar = least significant difference ($P \leq 0.05$).

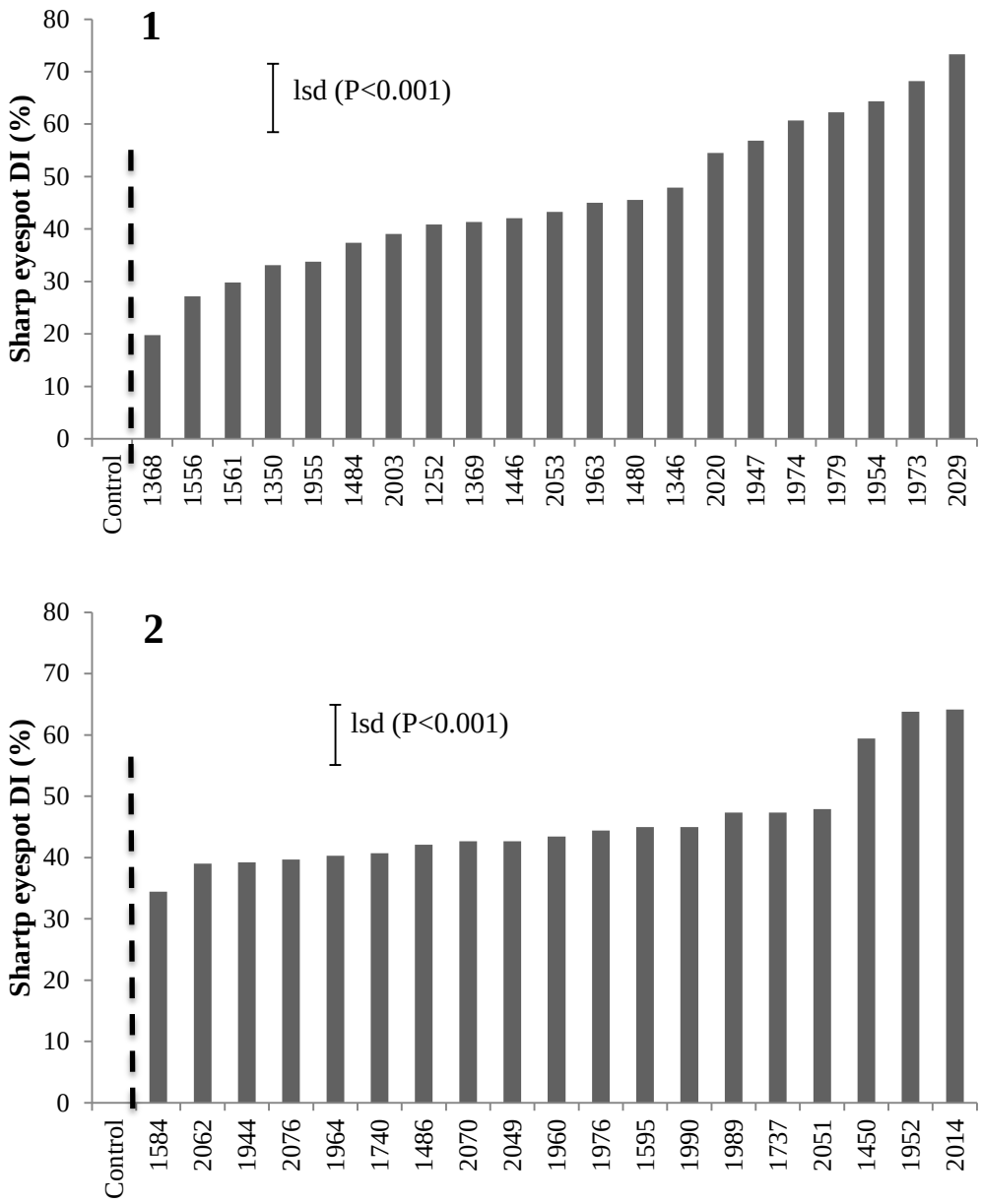


Figure 6.4. Sharp eyespot disease index (DI%) for 10 day old winter wheat (cv. Santiago) seedlings inoculated with *R. cerealis* isolates conducted over two experiments. 1 = experiment 1 and 2 = experiment 2. Dotted line represents the approximate point where sharp eyespot of the inoculated became significantly more ($P < 0.05$) than for the non-inoculated control. Bar = least significant difference ($P \leq 0.05$). Data is angular-transformed.

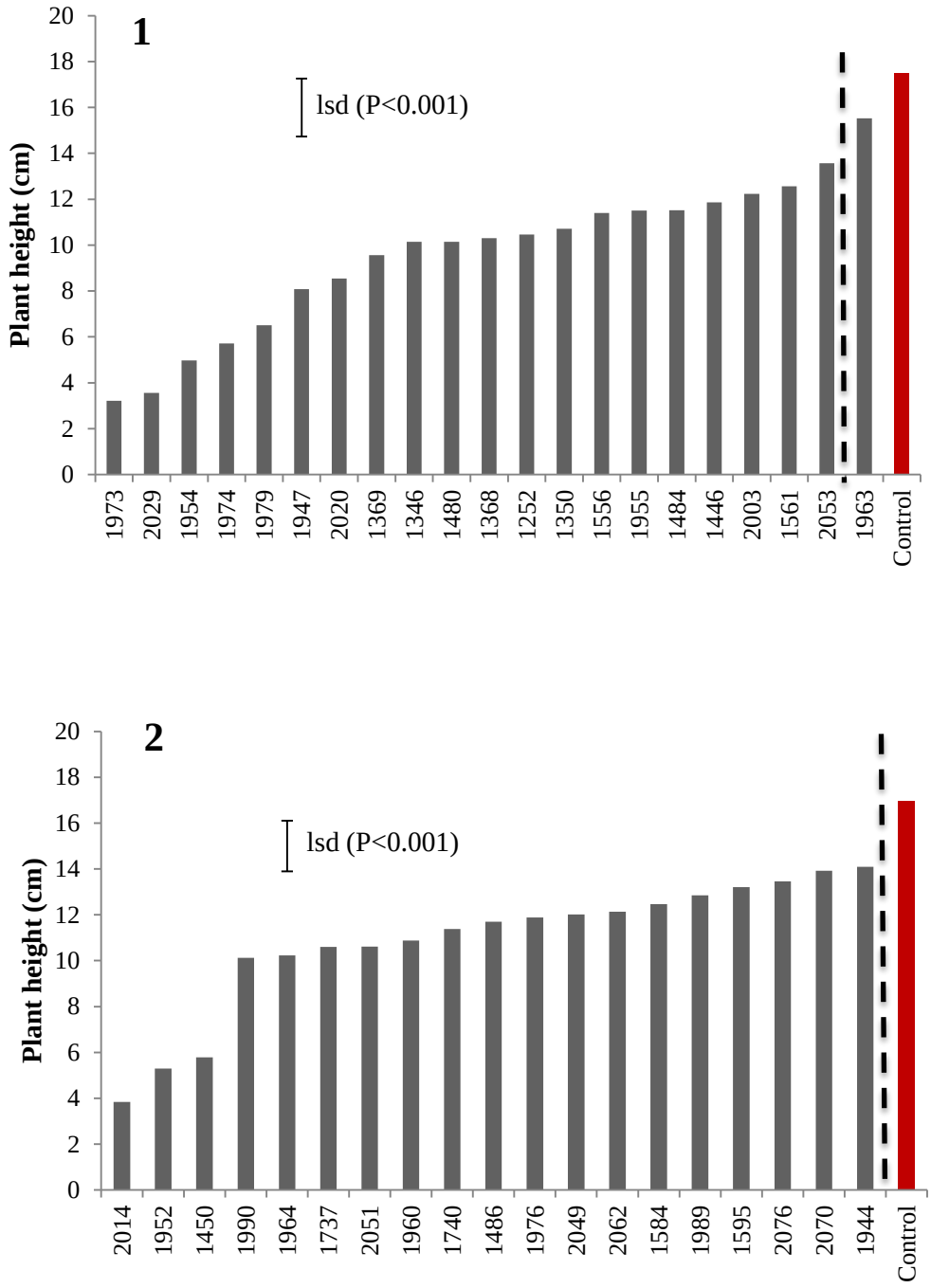


Figure 6.5. Plant height (cm) of 10 day old winter wheat (cv. Santiago) seedlings inoculated with *R. cerealis* isolates conducted over two experiments. 1 = experiment 1 and 2 = experiment 2. Dotted line represents the approximate point where plant height of the inoculated became significantly more ($P < 0.05$) than for the non-inoculated control. Bar = least significant difference ($P \leq 0.05$). Data is angular-transformed



Figure 6.6. Pre-emergence damping off caused by *R. cerealis* isolate 2029 (left) and the non-inoculated control (right) after 10 days growth.



Figure 6.7. Stunted development of 10 day old wheat seedlings inoculated with *R. cerealis* isolate 1486 (left) and the non-inoculated control (right).

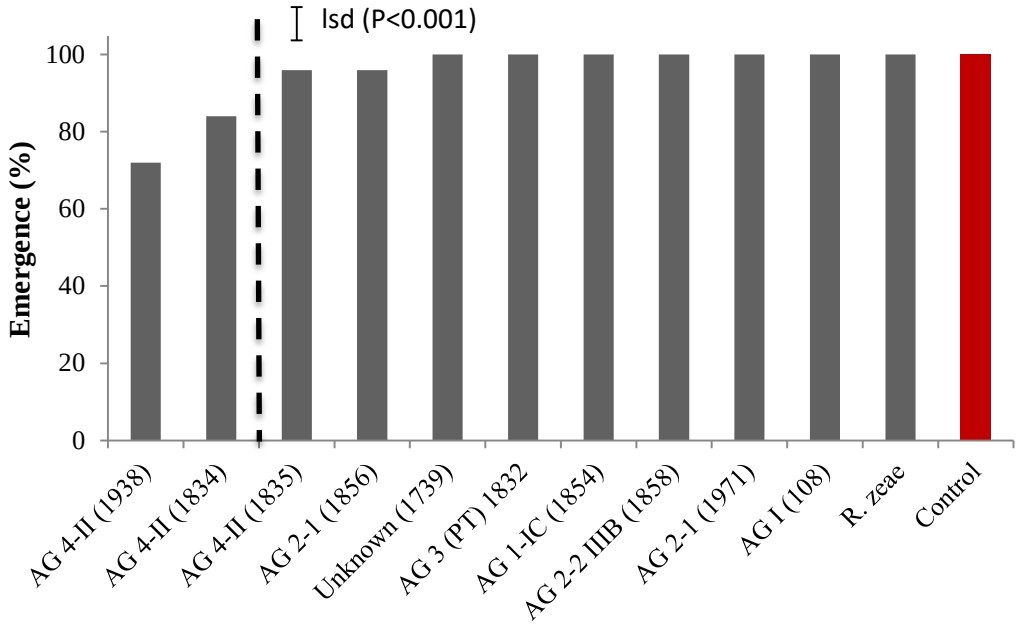


Figure 6.8. Emergence (%) of pre-germinated winter wheat (cv. Santiago) inoculated with *Rhizoctonia* spp. 10 days post planting in experiment 4. Dotted line represents the approximate point where emergence of the inoculated became significantly less ($P < 0.05$) than for the non-inoculated experimental control (red line). Bar = least significant difference ($P \leq 0.05$). Data is angular-transformed.

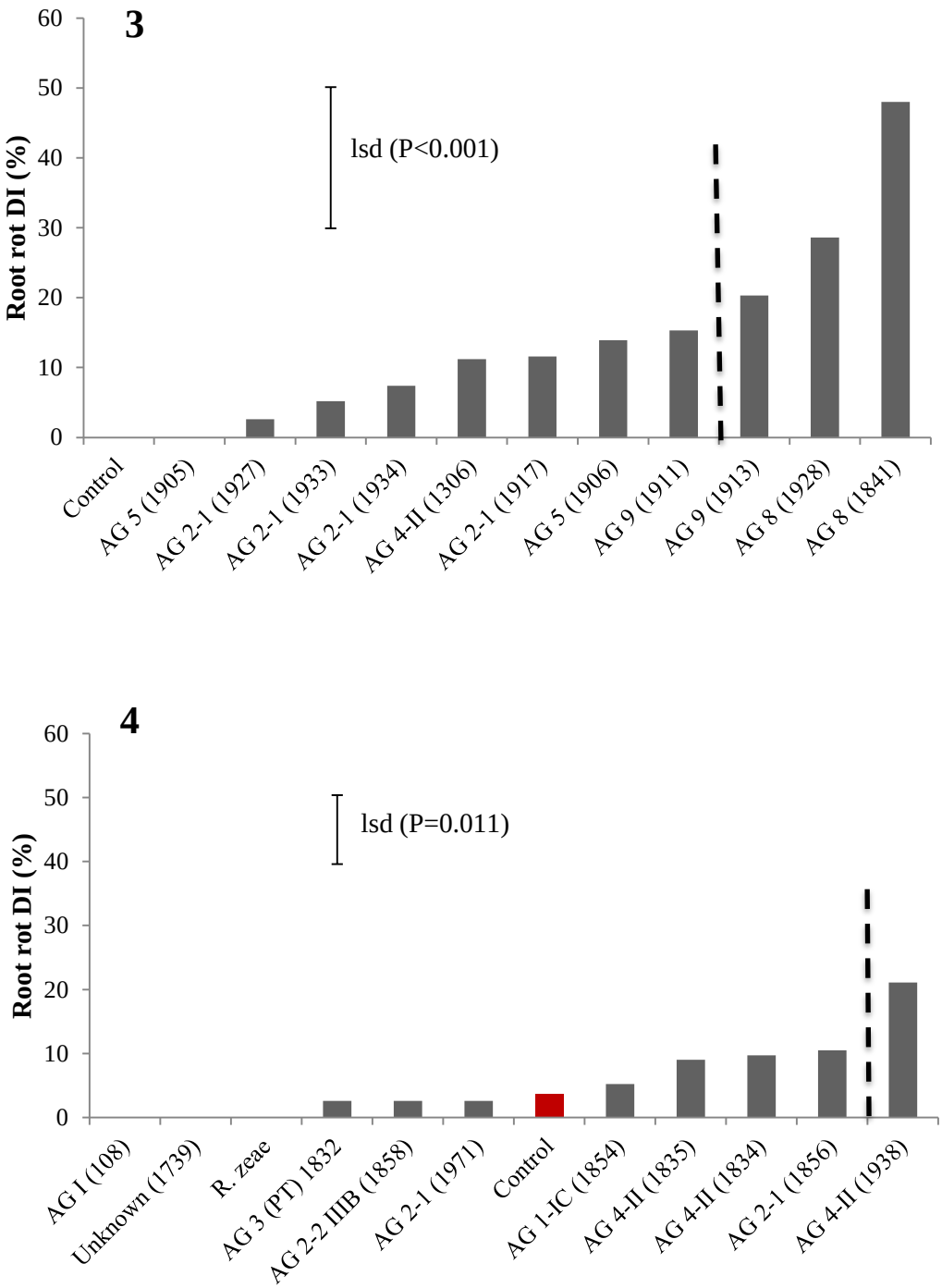


Figure 6.9. Root rot disease index (DI%) of 10 day old winter wheat (cv. Santiago) inoculated with *Rhizoctonia* spp. 3 = experiment 3 and 4 = experiment 4. Dotted line represents the approximate point where root rot of the inoculated became significantly less (P <0.05) than the non-inoculated experimental control (red line). Bar = least significant difference (P≤0.05). Data is angular-transformed.

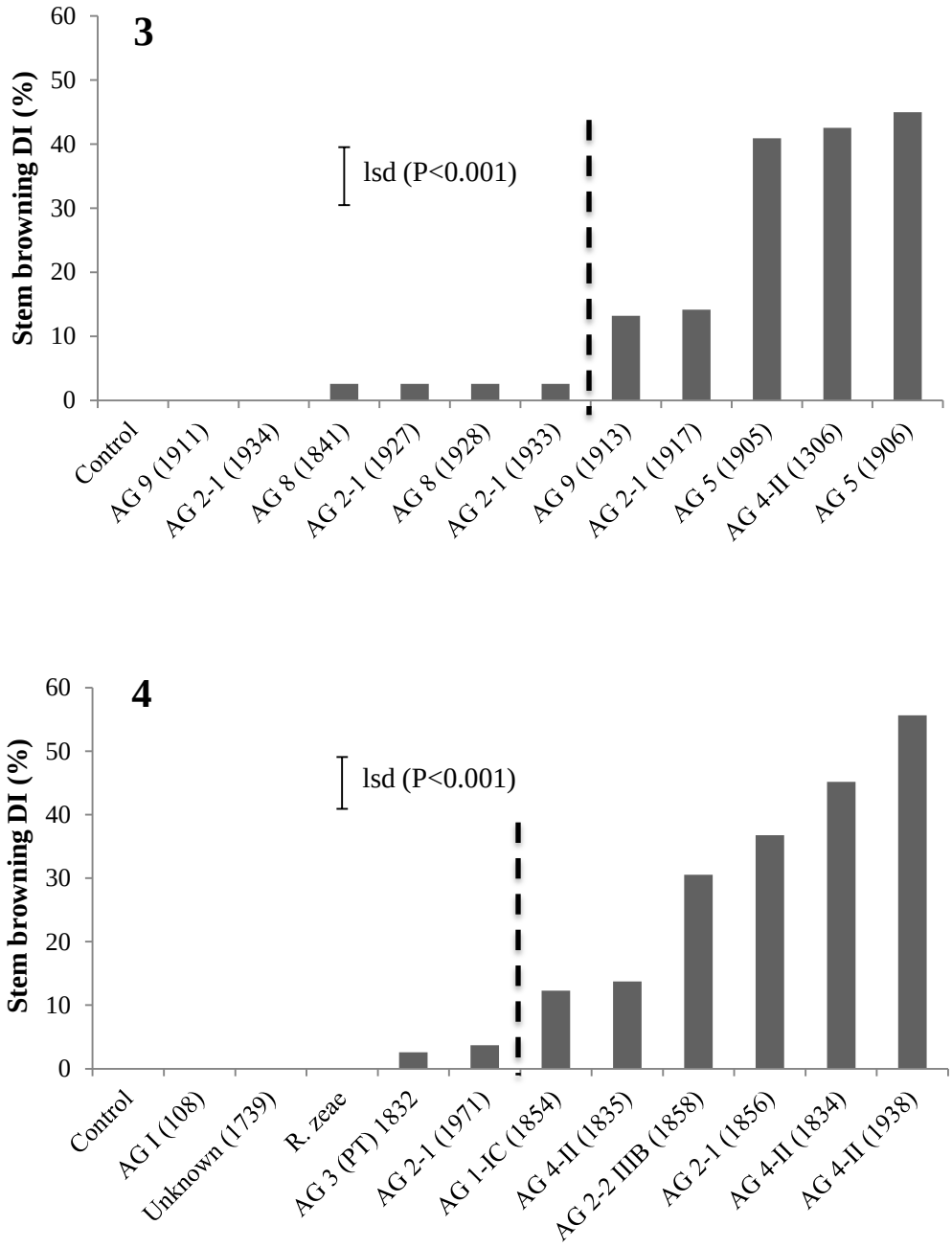


Figure 6.10. Rhizoctonia stem browning disease index (DI%) of 10 day old winter wheat (cv.. Santiago) inoculated with *Rhizoctonia* spp. 3 = experiment 3 and 4 = experiment 4. Dotted line represents the approximate point where stem browning of the inoculated became significantly less ($P < 0.05$) than for the non-inoculated experimental control (red line). Bar = least significant difference ($P \leq 0.05$). Data is angular-transformed.

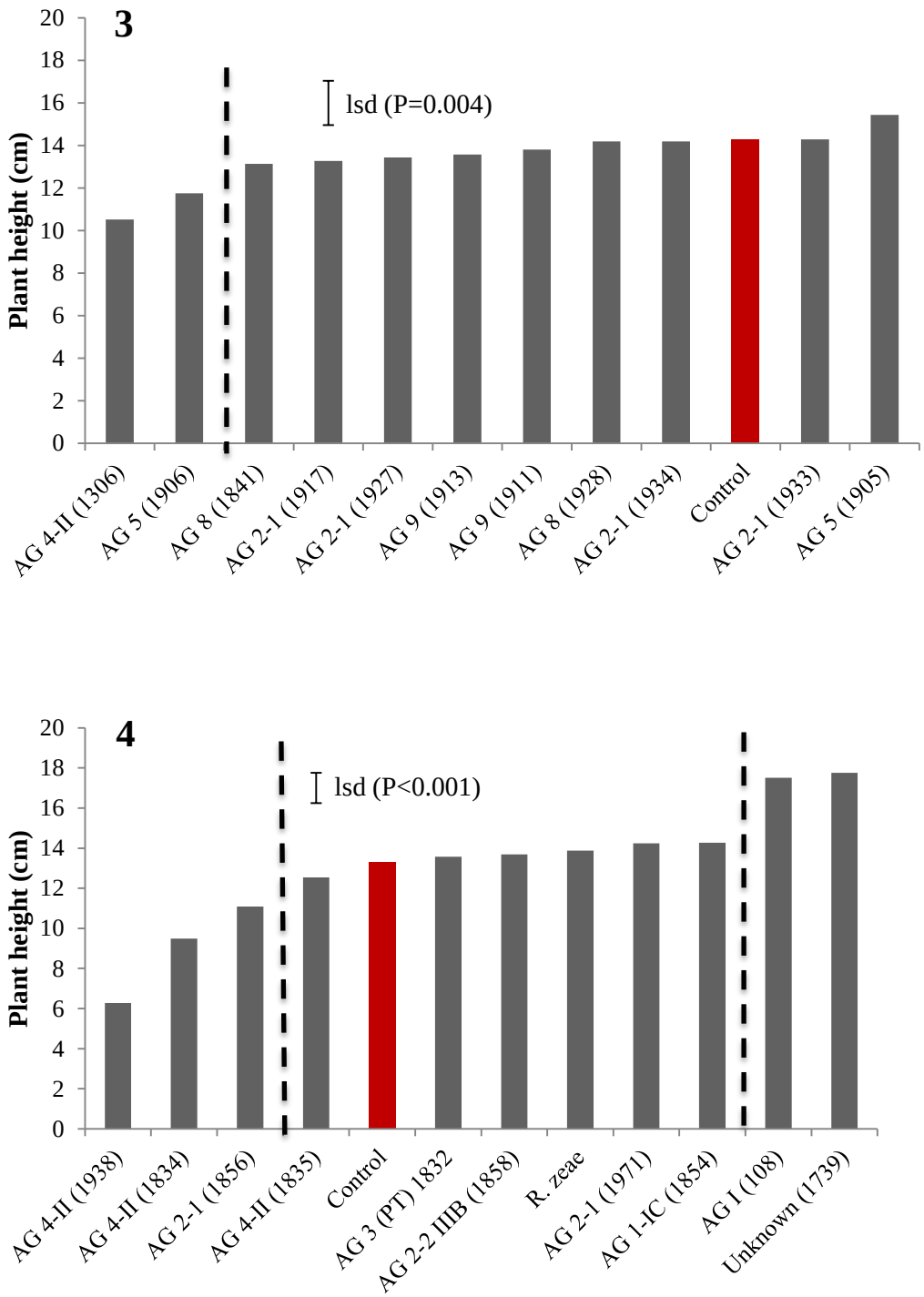


Figure 6.11. Plant height of 10 day old winter wheat (cv. Santiago) inoculated with *Rhizoctonia* spp. 3 = experiment 3 and 4 = experiment 4. Dotted line represents the approximate point where plant height of the inoculated became significantly ($P < 0.05$) lesser/greater than for the non-inoculated experimental control (red line). Bar = least significant difference ($P \leq 0.05$). Data is angular-transformed.

6.3.7 Discussion

This is the first study to investigate the pathogenicity of a range of *R. cerealis* isolates towards wheat seedlings. Isolates caused pre-emergence significant damping-off and lesions on the stems that resulted in reductions in plant height. However, there was large variation in the aggressiveness of isolates, for example disease on the stems ranged from superficial brown lesions to total maceration of the developing coleoptile. This study showed that 14 of the 40 isolates screened over two experiments were able to cause significant pre-emergence damping-off. However, there was large variation in emergence, ranging from 28% (isolate 2029) to 80% (2020) in experiment 1 and from 40% (2014) to 84% (1990) in experiment 2. This is the first study to show the difference in aggressiveness between isolates of *R. cerealis* causing pre-emergence damping-off. Previously Pitt *et al.* (1966) showed in glasshouse studies, from one isolate, pre-emergence losses between 7.6 and 45.4% over a range of inoculum densities. However, Burpee *et al.* (1980b) in growth chambers found six isolates of *R. cerealis* caused no reductions in emergence. Generally *R. cerealis* is associated with the stem disease sharp eyespot and therefore awareness of losses due to pre-emergence damping-off amongst agronomists and growers will be low. The results in this study suggest this pathogen can cause serious losses at the establishment phase. However, the inoculum levels in this trial will be considerably higher than those found in wheat fields. Therefore, field losses equivalent to those found in this study are unlikely to occur.

Root rot symptoms were observed from *R. cerealis* isolates in experiment 1 and 2 but these were not significant ($P<0.05$). Previous research has shown that isolates taken from sharp eyespot lesions did not cause root rot symptoms on wheat plants (Pitt, 1964; Lipps and Herr, 1982). The results from this experiment and previous studies suggests that *R. cerealis* is not a major pathogen of wheat roots. However, in both experiments all 40 *R. cerealis* isolates were able to cause significant stem browning compared to the control on 10 day-old seedlings, thus showing the ability of this pathogen to cause substantial disease at the seedling stage of development. The severity of sharp eyespot has been associated with yield losses (Clarkson and Cook, 1983; Lemańczy and Kwaśna, 2013). It is generally accepted that moderate to severe infections can cause significant yield

losses. In this study plants were not grown to maturity yet all but one isolate cause significant reduction in plant height. Therefore, early infections can lead to stunted development. However, previous studies have shown plants with early infections, resulting in stunted development, later recover due to the emergence of later formed healthy tillers from infected plants (Pitt *et al.,* 1966).

The other *Rhizoctonia* spp. and various AGs of *R. solani* had contrasting effects on wheat seedlings. The most aggressive isolates were those that belonged to AG 4-II, causing pre-emergence damping-off, root rot, stem browning and reductions in plant height. Tewoldmedhin *et al*. (2006) also found AG 4-II to cause significant damping off and coleoptile rot on 14 day old wheat seedlings but also on two other important crops, barley and canola. However, although in experiment 3 and 4 AG 4-II isolates caused disease on the stems there was variation between the isolates. Isolate 1938 in experiment 4 was the most aggressive causing emergence losses, root and stem disease and reduced height of surviving plants. *Rhizoctonia solani* AG 4-II is present in the UK but its distribution is limited. However, the AG 4-II isolates used in this study were all foreign isolates therefore it is not known what the pathogenicity of UK isolates is towards wheat. Reports of the pathogenicity of AG 2-1 towards wheat are contrasting with some studies showing the species is pathogenic (Roberts and Sivasithamparam, 1986) and other it is non-pathogenic (Khangura *et al.* 1999). This study showed that AG 2-1 isolates had no effect on seedling emergence or root rot. However, two isolates (1917 in experiment 3 and 1856 in experiment 4) were able to cause significant stem browning and one isolate (1856) reduced plant height compared to the control. These results suggest wheat is not a primary host for AG 2-1 which has been previously confirmed by others (e.g. Khangura *et al*. 1999; Oros *et al.* 2013). In experiment 4 AG 2-2-IIIB also caused stem browning. Previous studies have shown contrasting effects of this pathogen. Rush *et al.* (1994) showed AG 2-2 caused only mild symptoms but this only on the roots of wheat. *Rhizoctonia solani* AG 5 isolates also caused stem browning. Previously AG 5 isolated from wheat plants in Shropshire, England have also shown to cause lesions on the stems of wheat (Woodhall *et al.* 2012b). One of the AG 5 isolates (1906) in experiment 3 also reduced plant height showing this pathogen can effect seedling development. *Rhizoctonia solani* AG 8 is generally

considered the main AG of *R. solani* causing losses in cereals (Weller *et al.*, 1986; Mazzola *et al.*, 1996). In experiment 3 the two isolates of AG 8 caused lesions on the roots but no pre-emergence damping-off or stem browning, thus confirming this pathogen primary attacks the root system of wheat. However, no pruning of the root system was observed, this is probably due to the short time period (10 days) of this study. There was no effect of AG 8 on emergence in this study and this has also been previously shown by Mazzola *et al.* (1996). The most pathogenic AG 8 isolate (1841) on roots was cultured from wheat in Australia. In Australia this pathogen is considered a serious problem in wheat production causing the classic 'bare patch' symptoms within fields (Weller *et al.*, 1986). The other AG 8 (1928) isolate was cultured from UK soil and caused some root rot but was less aggressive than the Australian isolate. The difference in pathogenicity maybe due to the environmental conditions both these isolates evolved under. In addition, the full aggressiveness of these isolates may not have been shown under the temperature used in this study of 18°C. A previous study has shown AG 8 to cause only moderate disease at 20°C but severe root rot at 10°C (Ogoshi *et al.* 1990). *Rhizoctonia solani* AG 9 is generally considered only a pathogen of crucifers and potatoes (Carling *et al.*, 1987) and no previous studies have shown this species causes disease in wheat (Carling *et al.* 1987; Yang *et al.* 1996). To this authors knowledge this is the first report of AG 9 causing disease in wheat. However, only one (1913) of the two AG 9 isolates caused any significant disease and symptoms on roots and stems were relatively minor compared to those observed for AG 8 (roots) and AG 4-II (stems). The binucleate species AG-I in experiment 4 was found to be non-pathogenic. Demirci (1998) isolated AG I from wheat in Turkey also found this AG to be non-pathogenic. The results show that the various *R. solani* AG's in this study had contrasting effects on wheat seedlings. Although *R. solani* is generally associated with damping-off and root rot in wheat it was stem disease that was the most common symptom observed in this study.

.

6.4 Sensitivity of *Rhizoctonia* spp. to fungicides

6.4.1 Introduction

The sensitivity of both *R. solani* and *R. cerealis* isolates to several fungicides can vary (Carling *et al.,* 1990; Kataria and Gisi, 1989; Olaya *et al.,* 1994; Smiley *et al.,* 1990; Campion *et al.,* 2003; Chen *et al.,* 2014; Zhang *et al.,* 2015). The differences in fungicide sensitivity can be due to two mechanisms. Firstly, resistance from selection pressure of repeated application of the same fungicide. This has been shown with tolclofos-methyl resistance in *R. solani* and fludioxonil resistance in *R. cerealis* (van Bruggen and Arneson, 1984; Xia *et al.,* 2012). Secondly, natural insensitivity towards fungicides can occur between and within *Rhizoctonia* spp. For example pencycuron controlled a range of the *R. solani* AG's but had no effect on *R. cerealis* isolates (Kataria and Gisi, 1989). Natural insensitivity can also occur within isolates of the same AG. Tolclofos-methyl, fludioxonil and iprodione all controlled six isolates of AG 2-2, but control by pencycuron and benomyl showed considerable variation between isolates (Olaya *et al.,* 1994). However, the sensitivity of *R. cerealis* isolates towards fungicides has shown less variation (Sun *et al.,* 2015; Zhang *et al.,* 2015). Nevertheless, resistance in *R. cerealis* has been demonstrated *in vitro* experiments with fludioxonil and thifluzamide (Xia *et al.,* 2012; Zhang *et al.,* 2015). These reports show AG's can vary in there sensitivity which could lead to the selection of populations that are tolerant/resistant to certain fungicides.

The objective of this study was to:

i) To investigate the *in vitro* sensitivity of a range of *R. solani* and *R. cerealis* isolates towards two current and commonly used (fludioxonil and prothioconazole) and one new (sedaxane) seed treatments.

The specific null hypothesis tested within this study is:

i) *Rhizoctonia solani* and *R. cerealis* will show no *in vitro* sensitivity to fungicides (fludioxonil, prothioconazole and sedaxane).

6.4.2 Materials and methods

6.4.3 Isolates and fungicides

The experiment investigated the sensitivity of *Rhizoctonia* spp. towards three fungicides. Twenty five *R. cerealis* isolates used in this study were cultured from wheat plants at GS 21-31 in 2012. These isolates were chosen as they originated from eight distinct counties and crops in England and therefore were likely to reveal any difference in the sensitivity of current isolates towards fungicides. Appendix 10 lists the details of all isolates used in this study.

Technical grade fludioxonil (500 g a.i l^{-1}) and sedaxane (500 g a.i l^{-1}) (Syngenta Crop Protection, Schaffhauserstrasse, Stein, Switzerland) and commercial grade prothioconazole (250 g a.i l^{-1}) (Bayer CropScience) were diluted in analytical grade methanol (CH_3OH ≥99.9%) to make stock solutions of the required dilution series and stored at 4^{o}C for no more than 24 h. Methanol was added to media of the controls at a concentration of 0.5% (vol/vol) at the same volume as the fungicides throughout this study. Fungicide details are presented in Table 6.2.

6.4.4 Sighting experiment

The final fungicide concentrations chosen to assess the EC_{50} of the fungicides were determined empirically with an initial sighting test. Sighting concentrations were 0.5, 0.05, 0.005, 0.0005, 0.00005 mg a.i l^{-1} for fludioxonil and sedaxane and 0.8, 0.08, 0.008, 0.0008, 0.00008 mg a.i l^{-1} for prothioconazole. Two isolates of *R. solani* AG 2-1 (1917 & 1933), AG 5 (1905 & 1905) and *R. cerealis* (1480 & 1561) were the test AG's. For the method and analysis refer to the following two sections below.

6.4.5 Sensitivity of *Rhizoctonia* spp. to fludioxonil, prothioconazole and sedaxane

PDA (39 g l^{-1}) was autoclaved at 121^{o}C for 15 minutes then allowed to cool to 50^{o}C before fungicides were added in seven 10-fold dilutions, resulting in the final concentrations: 0.5, 0.25, 0.125, 0.063, 0.031, 0.016 or 0.008 mg a.i l^{-1} for fludioxonil and sedaxane, 0.7, 0.35, 0.175, 0.088, 0.044, 0.022 or 0.011 mg a.i l^{-1} for prothioconazole. Amended PDA was added to 9 cm Petri dishes (Sterilin™

Standard). Plates without fungicides but with an equivalent volume of methanol were included as controls. Each dish was inoculated with a 5 mm diameter plug taken from the edge of a 6-7 day old plate of an appropriate isolate. Plugs were positioned in the centre of the dish. Three replicate dishes of each isolate and fungicide concentration were incubated at 20^{o}C in the dark until the growth of the controls had reached approximately 7-8 cm diameter (usually 4-7 days depending on AG). Isolate growth was measured at two perpendicular angles and the average of the two measurements used for analysis. The 5 mm plug was subtracted from the average diameter.

6.4.6 Statistical analysis

All data was analysed using Genstat® Version 16th Edition for Windows (VSN International Ltd, UK). To calculate the effective concentration to reduce fungal growth by 50% (EC_{50}) follows: Colony growth for each fungicide concentration was converted to a percentage of the control of each isolate with the following equation:

Percentage growth = (colony diameter * 100)/control diameter

Percentage growth was then converted into a proportion of the control:

Proportion = (100 – percentage growth)*100 (control)

Fungicide dose was then $\log_{10}$ transformed. EC_{50} estimates could then be made for each *Rhizoctonia* spp. isolate towards each fungicide concentration by logit analysis.

Table 6.2 Fungicide name, class, mode of action and chemical structure

Fungicide	Class*	Mode of action
Fludioxonil	Phenylpyrrole	Inhibits transport-associated phosphorylation of glucose, reducing mycelial growth.
Prothioconazole	DMI-fungicides (Demethylation Inhibitors)	Interference with the synthesis of ergosterol in the target fungi. Resulting in changes to the fungal cell membrane.
Sedaxane	SDHI (Succinate dehydrogenase inhibitors)	Inhibits respiration by binding to the succinate dehydrogenase complex in the mitochondria.

* Fungicide resistance action committee (FRAC) classification

6.4.7 Results

A total of 25 field collected *R. cerealis* isolates were tested for the sensitivity to prothioconazole, fludioxonil and sedaxane during the mycelial growth stage. Isolates were generally more sensitive to sedaxane and fludioxonil than prothioconazole (Table 6.3). Sedaxane had the lowest mean EC_{50} (MEC_{50}) of 0.032 mg a.i l^{-1} this was followed by fludioxonil (0.036 mg a.i l^{-1}) and prothioconazole (0.044 mg a.i l^{-1}). The range of EC_{50} values was larger for sedaxane and prothioconazole compared to fludioxonil. This indicates that isolates showed a greater range of sensitivity towards sedaxane and prothioconazole than fludioxonil.

A total of 25 *R. solani* isolates were tested for the sensitivity to prothioconazole, fludioxonil and sedaxane (Table 6.4). Sedaxane had the lowest MEC_{50} of 0.044 mg a.i l^{-1} this was followed by fludioxonil (0.103 mg a.i l^{-1}) and prothioconazole (0.151 mg a.i l^{-1}). The MEC_{50} values for sedaxane were at comparable values to those found for isolates of *R. cerealis*. The MEC_{50} for prothioconazole was 3.4-fold higher for *R. solani* isolates than those of *R. cerealis*. However, the range of each EC_{50} values for each fungicide was highly variable. Generally sedaxane had the lowest EC_{50} values for each isolate. However, one isolate of AG 4-II (1834) showed a high degree of tolerance towards sedaxane (Figure 6.14). Prothioconazole range of EC_{50} values were from 0.010 to 0.382 mg a.i l^{-1}, with the one isolate of AG 11 the most tolerant and AG 3 (1902) the most sensitive. Also relevantly tolerant with EC_{50} >0.10 mg a.i l^{-1} were some isolates of AG 2-1, 4-II, 5 and 8. Fludioxonil showed the largest range of EC_{50} values from 0.014 to 1.65 mg a.i l^{-1}. The large range for fludioxonil was due to isolate 1739, a potentially new *Rhizoctonia* spp., which was relatively insensitive to fludioxonil but was well controlled by sedaxane (Figure 6.15).

Variability within isolates of the same AG was also shown. The largest variability in EC_{50} values for each fungicide occurred with isolates of AG 3; fludioxonil, sedaxane and prothioconazole showed 3.2-, 5.3-, and 10.4-fold differences between the lowest and highest EC_{50} values respectively.

Table 6.3. EC_{50} values of prothioconazole, fludioxonil and sedaxane for a range of *R. cerealis* isolates (*n* = 25) cultured from wheat plants in 2012 and 2014.

	EC_{50} (mg a.i l^{-1})		
Isolate	**Prothioconazole**	**Fludioxonil**	**Sedaxane**
1252	0.040	0.043	0.027
1314	0.046	0.048	0.050
1369	0.061	0.036	0.047
1595	0.040	0.036	0.029
1605	0.060	0.038	0.050
1740	0.062	0.033	0.045
1346	0.024	0.034	0.031
1350	0.032	0.028	0.028
1368	0.057	0.031	0.036
1484	*	0.032	0.060
1435	0.048	0.042	0.025
1448	0.042	0.045	0.022
1451	0.044	0.031	0.025
1480	0.027	0.037	0.031
1486	0.042	0.028	0.022
1510	0.035	0.029	0.026
1450	0.043	0.036	0.018
1514	0.053	0.038	0.041
1556	0.014	0.036	0.021
1446	0.046	0.029	0.026
1561	0.059	0.032	0.046
1726	0.039	0.031	0.026
1737	0.036	0.040	0.020
1584	0.046	0.045	0.032
1585	0.05	0.043	0.028
Range	0.014 to 0.062	0.028 to 0.048	0.018 to 0.060
MEC_{50}	0.044	0.036	0.032
SD	0.012	0.006	0.011
SE	0.003	0.001	0.003
CV (%)	27.53	16.00	34.65

* indicates no data available

MEC_{50} = mean effective concentration to reduce hyphal growth by 50%

Table 6.4. EC_{50} values of prothioconazole, fludioxonil and sedaxane for a range of anastomosis groups of *R. solani* isolates.

		EC 50 (mg a.i l^{-1})		
AG	**Isolate**	**Prothioconazole**	**Fludioxonil**	**Sedaxane**
AG 2-1	1917	0.121	0.028	0.040
AG 2-1	1926	0.186	0.031	0.057
AG 2-1	1927	*	*	0.019
AG 2-1	1933	0.090	*	0.021
AG 2-1	1934	0.055	0.019	0.017
AG 2-1	1935	0.231	0.025	0.022
AG 2-1	1942	0.055	0.031	0.019
AG 3	1900	*	0.034	0.065
AG 3	1901	0.104	0.025	0.016
AG 3	1902	0.010	0.079	0.071
AG 3	1904	0.019	0.062	0.081
AG 3	1920	0.050	0.079	0.085
AG 4-II	1834	0.051	0.015	0.210
AG 4-II	1924	0.123	*	0.041
AG 4-II	1937	0.319	*	0.046
AG 5	1905	0.334	0.025	0.024
AG 5	1906	0.238	0.026	0.024
AG 8	1907	0.309	0.037	0.090
AG 8	1928	0.339	0.018	0.093
AG 9	1908	0.109	0.029	0.012
AG 9	1909	0.088	0.016	0.014
AG 9	1911	0.163	0.036	0.032
AG 9	1912	0.080	0.039	0.013
AG 9	1913	0.094	0.024	0.020
AG 9	1914	0.123	0.014	0.017
AG 11	1915	0.382	0.032	0.035
Unknown	1739	0.110	1.646	0.013
Range		0.010 to 0.382	0.014 to 1.65	0.012 to 0.210
MEC_{50}		0.151	0.103	0.044
SD		0.11	0.34	0.04
SE		0.02	0.07	0.01
CV%		72.68	327.08	95.33

* indicates no data available

MEC_{50} = mean effective concentration to reduce hyphal growth by 50%

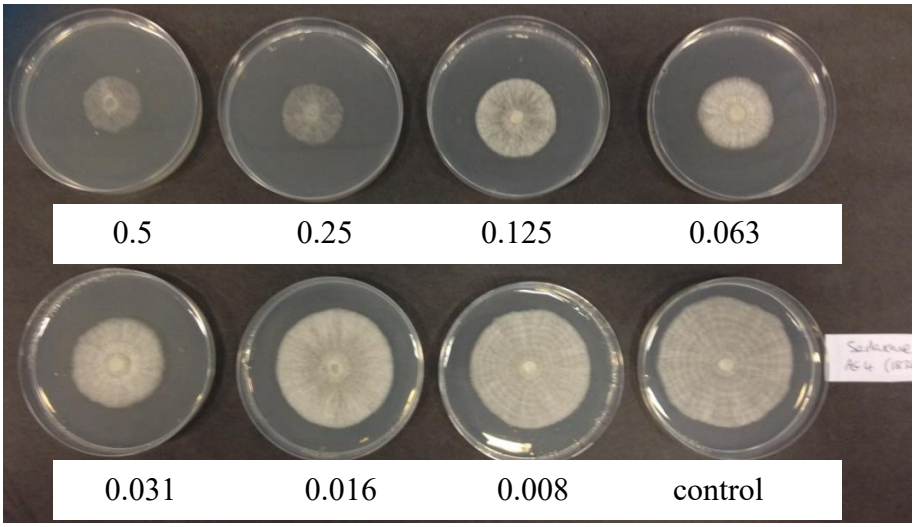


Figure 6.12. Growth of AG 4-II (1846) on sedaxane amended plates in seven 10-fold dilutions from 0.5 ppm to 0.008 ppm and a control amended with methanol (MeOH).

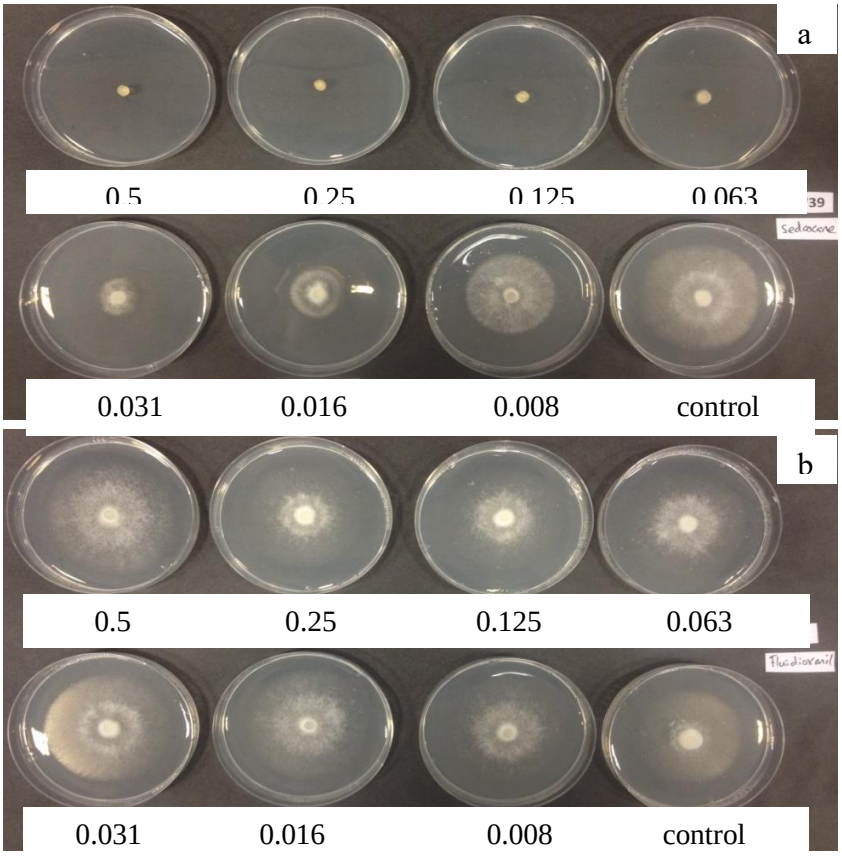


Figure 6.13. Growth of isolate 1739 on (a) sedaxane and (b) fludioxonil amended plates in seven 10-fold dilutions from 0.5 ppm to 0.008 ppm and a control amended with methanol (MeOH).

6.4.8 Discussion

The MEC_{50} values of prothioconazole, fludioxonil and sedaxane for 25 *R. cerealis* isolates were all of the same order. Sedaxane had the lowest MEC_{50} (0.032 mg a.i l-1) value of the three fungicides. Previous studies have shown EC_{50} values for sedaxane for *R. cerealis* were 0.07 mg a.i l^{-1} (Zeun *et al.,* 2013) and 0.058 mg a.i l^{-1} (Goll *et al.,* 2014). However, these two studies only screened one isolate and therefore not a true reflection of the population. Fludioxonil showed the smallest range of EC_{50} values thus providing the most consistent control of *R. cerealis*. Hamada *et al.* (2011b) found MEC_{50} for fludioxonil to control 89 *R. cerealis* isolates cultured from wheat crops in China was 0.033 mg a.i l^{-1}. Therefore the MEC_{50} of 0.032 mg a.i l^{-1} (sedaxane) and 0.036 mg a.i l^{-1} (fludioxonil) were of the same order as ones previously published for *R. cerealis*. As 25 isolates were assessed in this study it provides a more accurate reflection of the MEC_{50} of the *R. cerealis* population. To this authors knowledge there are no previous published prothioconazole EC_{50} values for *R. cerealis* to compare with this study. However, the data suggests that prothioconazole offers comparable control to that of fludioxonil and sedaxane *in vitro* tests.

Rhizoctonia solani isolates were most sensitive to sedaxane (MEC 0.044 mg a.i l^{-1}), followed by fludioxonil (MEC 0.103 mg a.i l^{-1}) prothioconazole (MEC 0.151 mg a.i l^{-1}). However, the MEC_{50} for fludioxonil would have been lower but for the relative insensitivity of isolate 1739. This isolate is potentially a new *Rhizoctonia* spp. Although found to be non-pathogenic on wheat seedlings in this study (section 6.3), the pathogenicity, if any, to other species is not known. In this study *R. solani* isolates were least sensitive to prothioconazole. Demethylation inhibitors are known to have highly variable actively towards AG's of *R. solani.* This could be due to different uptake and accumulation in fungal cells, greater capacity to metabolise the fungicide, or molecules have difficulty binding to the target site (Kataria and Gisi, 1999). A previous study has shown prothioconazole EC_{50} values for *R. solani* ranged from 0.20 to 5.58 mg a.i l^{-1} with a mean of 1.74 mg a.i l^{-1} (Ajayi and Bradley, 2014). Therefore, the MEC_{50} in this study (0.151 mg a.i l^{-1}) was one order of less than those of Ajayi and Bradley, (2014). However, caution is required in the interpretation of the MEC_{50} data as this is an overall mean for all AG's of *R. solani* in this study. Therefore this does not take

account of the sensitivity of individual AGs towards each fungicide. The activity of fungicides to specific AGs of *R. solani* is important when specific AGs need to be targeted.

One notable isolate, AG 4-II (1834), showed relative tolerance towards sedaxane with an EC_{50} value approximately one order higher than for other isolates. This was also the most aggressive *R. solani* isolate towards wheat seedlings in this study. Insensitivity can suggest a risk of resistance developing. A further experiment should be conducted growing this isolate over repeated generations on sedaxane amended PDA to monitor any changes in sensitivity. Previous sedaxane EC_{50} values have ranged from <0.01 to 0.03 mg a.i l^{-1} (Zeun *et al.*, 2013), 0.02 to 0.043 mg a.i l^{-1} (Goll *et al.*, 2014) and 0.02 to 0.49 mg a.i l^{-1} (Ajayi and Bradley, 2014) for a range of *R. solani* isolates. Fludioxonil is also known to provide good control of various AG's of *R. solani.* Djébali *et al.* (2014) found the fludioxonil MEC_{50} for AG 3 isolates cultured from potatoes in Tunisia was 0.007 μg a.i. ml^{-1}. Meyer *et al.* (2006) recorded an EC_{50} of 0.003 mg a.i l^{-1} for AG 1-IA. While an earlier study by Olaya *et al.* (1994) shows that fludioxonil at 0.1 μg a.i. ml^{-1} only inhibited growth of AG 2-2, AG 4 and AG 5 but showed total control at concentrations of 10 and 100 μg a.i. ml^{-1}. Several AG's can infect a plant at any one time, therefore fungicides used to control *Rhizoctonia* spp. should be active against all AG's in any given disease situation (Kataria and Gisi, 1999). The results from this study show that of the three fungicides fludioxonil and sedaxane offered the most consistent control over a range of AG's.

6.5 Conclusions

The characterisation of *Rhizoctonia* isolates can provide information to improve control measures of these species. This was achieved in this study by a) providing greater understanding of the population structure of *R. cerealis*, b) observing the pathogenicity of a range of *Rhizoctonia* isolates towards winter wheat seedlings and c) revealing the sensitivity of AG's towards two commonly used (prothioconazole and fludioxonil) and one new (sedaxane) seed treatments.

The results in this chapter enable the following conclusions to be made:

i) Twenty four unique ITS regions of *R. cerealis* isolates cultured from English wheat crops were identified.

ii) All 160 *R. cerealis* isolates belonged to AG D-I. This is the first study to show the main sharp eyespot causing species in England is AG D-I.

iii) All *R. cerealis* isolates tested were pathogenic to wheat seedlings. However, considerable variation was observed in the aggressiveness between isolates.

iv) The most serious effect of *R. cerealis* was to cause pre-emergence damping-off with seven isolates reducing emergence to <55%.

v) *Rhizoctonia solani* AG's were observed to vary in pathogenicity to wheat seedlings. The most aggressive isolates causing reductions in emergence and stem disease were those of AG 4-II. *Rhizoctonia solani* AG 8 was the most aggressive AG towards roots.

vi) The binucleate species AG I potentially had a beneficial effect on growth of wheat seedlings.

vii) Prothioconazole, fludioxonil and sedaxane all showed comparable activity towards *R. cerealis* isolates. The results show *R. cerealis* is easily controlled by these three fungicides.

viii) Anastomosis groups of *R. solani* were more sensitive to fludioxonil and sedaxane than to prothioconazole.

Chapter 7

GENERAL DISCUSSION

7.1 GENERAL DISCUSSION

This study found the predominant AG of *R. solani* in English wheat fields was AG 2-1, occurring in 63% of soil samples (chapter 3). This is in contrast to the finding of Goll *et al.* (2014) that identified AG 9 as the dominant AG of *R. solani* in UK arable fields. The main agronomy practice influencing this pathogen in soil was previous crop with the highest concentrations occurring after OSR (chapter 4). This is in agreement with a previous study that showed the prior crops grown can influence the amount of AG 2-1 DNA in soil (Sparrow *et al.*, 2015). *Rhizoctonia solani* AG 2-1 is an aggressive pathogen of *Brassica* spp. that causes considerable damping-off and root rot (Verma, 1996; Babiker *et al.*, 2013; Sturrock *et al.*, 2015). Therefore growing OSR in shorter rotations is likely to select for AG 2-1 in the soil. In chapter 3 AG 2-1 DNA in soil or *in planta* was not influenced by environmental conditions. The lack of any environmental effect in this study, especially in the soil, is likely due to the short time period that meteorological data was collected. Although AG 2-1 had the highest biomass in soil samples it had the lowest pathogen DNA *in planta* (chapter 3). This suggests wheat is not a major host for AG 2-1. The results from the field experiments provided further evidence that AG 2-1 has insignificant effects on the growth of winter wheat in the UK. This should be treated with some caution as only one AG 2-1 isolate was investigated in the field experiment. In the controlled environment study two AG 2-1 isolates caused significant stem browning and one isolate reduced plant height of 10 day old wheat seedlings showing that there is variation in the pathogenicity of AG 2-1 isolates to wheat. Further field experiments would be necessary to determine the full extent to which a range of AG 2-1 isolates effects wheat. In addition, future studies should also carry out soil isolations from English wheat fields to investigate the difference in pathogenicity among AG 2-1 isolates to wheat but also OSR.

Classic rhizoctonia root rot symptoms of spear tipping and pruning were rarely seen in English wheats crops (chapter 3). This is probably because the dominant AG of *R. solani* identified in chapter 3 was AG 2-1 which is generally a weak pathogen of wheat. Further evidence that AG 2-1 causes only superficial symptoms on roots was provided in the field experiments (chapter 5) were AG 2-1 in addition to AG 5 and *R. cerealis* caused no significant root rot symptoms

above that seen in the non-inoculated control. Although, DNA of these species was detected on roots of wheat in the field experiments there were no correlations with root rot index or yield (t ha^{-1}) of winter wheat. There was also no or weak associations in the PCA between AG 2-1 or *R. cerealis* DNA in roots and root rot index in commercial wheat crops (chapter 3). These results suggest the impact on root health from AG2-1 is marginal in English wheat crops. The results from the controlled environment study showed the most pathogenic AG of *R. solani* to wheat roots was AG 8; notably isolate 1841 which was isolated from wheat in Australia. This supports previous research that AG 8 is the main AG causing rhizoctonia root rot in wheat (MacNish and Neate, 1996; Paulitz *et al.*, 2002). In chapter 3 AG 8 was detected infrequently in just 2% of wheat fields and therefore is unlikely to be a major problem in English wheat production. Nonetheless root rot symptoms were observed in both commercial wheats fields (chapter 3) and the field experiments (chapter 5). These symptoms were probably caused by a complex of species that reside in rhizosphere (Harris and Moen, 1985; Raaijmakers *et al.*, 2009). The predominant species identified on the roots of wheat in chapter 3 were the *Oculimacula* spp. This is the first study to quantify these species on the roots of wheat however there was no association between the *Oculimacula* spp. and root rot in the PCA in chapter 3. It is not known what role, if any, root infection plays in the epidemiology of *Oculimacula* spp. or if it has negative impact on crops.

In the field experiments root rot at GS 15 was significantly reduced by fludioxonil and sedaxane + fludioxonil compared to the untreated. There was an additional 6% reduction in root rot from sedaxane in mixture with fludioxonil. Indeed in chapter 6 sedaxane was highly active against all AGs of *R. solani*. In addition, all AGs of *R. solani*, except a potentially a new *Rhizoctonia* spp. (isolate 1739), were sensitive to fludioxonil. In contrast the efficiency of prothioconazole to AGs of *R. solani* was moderate with EC_{50} values generally one order higher than sedaxane and fludioxonil. Kataria and Gisi, (1999) stated that fungicides should ideally be active against all *Rhizoctonia* spp. and AGs. This shows the advantage of sedaxane and to some extent fludioxonil over prothioconazole as seed treatments to control *Rhizoctonia* spp. as generally growers will be unaware of which AGs are present in fields.

Rhizoctonia cerealis, the causal agent of sharp eyespot, is not generally considered a major yield reducing pathogen of wheat. Even so in the field experiments of this study *R. cerealis* caused significant reduction in emergence and grain yield (t ha^{-1}) of winter wheat. This shows *R. cerealis* can seriously impact on the development of wheat crops from emergence to grain yield. Therefore growers and agronomist should give this species greater attention as generally it is considered only a relatively minor pathogen. In chapter 3 the pathogen was detected in 54% of soil samples thus the majority of wheat crops are at risk of early infections from this species (chapter 3). In chapter 5 *R. cerealis* reduced emergence of untreated seed by 35% compared to the non-inoculated control in 2013/14. Further evidence of emergence losses was provided in chapter 6 were 14 out of 40 *R. cerealis* isolates significantly reduced the emergence of wheat seedlings. The pathogenicity experiment in chapter 6 showed isolates of *R. cerealis* were generally more aggressive than isolates of the various AGs of *R. solani,* causing greater damping-off and stem browning. That said in chapter 6 AGs (e.g. AG 4-II and AG 8) of *R. solani* were more aggressive towards the roots of wheat, and in chapter 5, AG 5 was able to cause significant stem browning in field conditions. In the field experiments sedaxane + fludioxonil increased emergence in *R. cerealis* inoculated plots by 54% compared to the untreated in 2013/14 but there was no response to fludioxonil alone. This showed an additional benefit of sedaxane in mixture with fludioxonil. The lack of response to fludioxonil is surprising. In chapter 6 EC_{50} values were of the same order for sedaxane, fludioxonil and prothioconazole showing each fungicide had similar control of *R. cerealis in vitro* tests. This suggests sedaxane performs better in field conditions at reducing damping-off caused by *R. cerealis* than fludioxonil alone.

Seedlings that survived damping-off caused by *R. cerealis* in chapters 5 and 6 showed signs of stunted development. This was controlled in chapter 5 by fludioxonil and sedaxane + fludioxonil treatments that both increased plant length through activity against the pathogen. In the field experiments sharp eyespot control at GS 31, 39 and 75 by seed treatments was inconsistent. This suggests foliar fungicide treatments are required to control sharp eyespot beyond GS 31. However, sedaxane + fludioxonil significantly reduced BFR severity at GS 31

and GS 39 compared to the untreated. Direct comparison with chapter 3 cannot be made as all crops were grown from treated seed. Even so it does show that sedaxane + fludioxonil treatments provided continued protection from BFR causing species up to GS 39 beyond the main period that seed treatments are generally considered active. Sharp eyespot index was generally higher in the field experiments than those shown in commercial wheat fields (chapter 3). This was most notable at GS 31 and is likely due to the placement of inoculum alongside the sown seed allowing earlier infections than occur naturally in commercial wheat fields. Therefore, the reduction of yield by 8.5 % in *R. cerealis* inoculated plots compared to the untreated may be higher than would be expected in naturally infested fields. Although these losses are similar to previous studies where fields naturally infested with *R. cerealis* have shown yield reductions of 5-10% (Clarkson and Cook, 1983; Lemańczyk and Kwaśna, 2013). Further investigation into the population structure of *R. cerealis* in English wheat crops was conducted by investigating the sequence heterogeneity of the rDNA-ITS region of *R. cerealis* isolates (chapter 6). Twenty four unique ITS regions were identified in 160 *R. cerealis* isolates collected from wheat plants across England. This study revealed that all 160 isolates belonged to AG D-I. This supports previous work by Li *et al.* (2014) in China that also showed the main *R. cerealis* subgroup causing sharp eyespot symptoms is AG D-I.

Stem browning is generally associated with *Microdochium* spp. but this study showed *Rhizoctonia* spp. also cause similar symptoms at the early growth stages in English wheat crops making identification of symptoms of individual diseases difficult. It is likely symptoms were indiscriminate when pathogens occurred in complexes of diseases, this was suggested in the grouping of sharp eyespot and BFR in the PCA (stem base GS 21-31). In the field experiments stem browning was consistently reduced by sedaxane + fludioxonil treatments in both seasons. In contrast fludioxonil alone was less consistent, only reducing stem browning in 2012/13 experiment. In commercial wheat fields (chapter 4) seed treatments did not influence SBDs or their associated casual agents. This could be due to a range factors, including; collecting samples at GS 21-31 would be considered beyond the main period seed treatments are active or the seed treatments were not active on the pathogens/diseases assessed in this study.

This study has shown a shift in the population dynamics of the *Oculimacula* spp. Previous studies have stated that *O. acuformis* is the dominant species (Turner *et al.*, 1999; Nicholson and Turner, 2000; Turner *et al.*, 2001). This study showed both species appeared together in crops but that *O. yallundae* was clearly the dominant species in terms of biomass. The *Oculimacula* spp. was detected on average in 98% of stem bases throughout the period of study (chapter 3). In chapter 3 eyespot was identified at GS 65-75 in 98% (2011/12) and 74% (2012/13) of crops. Therefore it can be stated that the *Oculimacula* spp. are ubiquitous in English wheat crops. PCA clearly grouped *O. acuformis* and *O. yallundae* together in soil and *in planta* at each sampling period indicating a strong association between these related species (chapter 3). It is suggested the use of species specific real-time PCR assays allowed for the first time an accurate picture of the population dynamics to be revealed. Despite this, eyespot severity and *Oculimacula* spp. development showed considerable variation between growth stages and seasons, caused primarily by environmental conditions but also agronomy practices (chapter 3 & 4). Chapter 4 identified that early sown crops and 2nd and continuous wheats favoured eyespot severity and *Oculimacula* spp. development more than late sown crops and 1st wheats. Regardless of this, the strongest influence on development of eyespot and *Oculimacula* spp. was environmental conditions. In the autumn/winter period low rainfall and milder temperatures favoured eyespot severity and *Oculimacula* spp. development (chapter 3 & 4). However, the strongest correlations were shown in the spring when eyespot severity and *Oculimacula* spp. DNA in stem material at GS 37-45 was positively associated with increased rainfall, humidity and mean atmospheric temperatures (chapter 3). PCA also grouped eyespot and meteorological factors at GS 37-45; but *Oculimacula* spp. generally showed independence from eyespot symptoms and meteorological factors (chapter 3). In chapter 4 fungicides applied at T1 (GS 30-31) and T2 (GS39) failed to influence eyespot severity in commercial wheat fields.

BFR in chapter 3 occurred in 100% of crops by GS 37 in both seasons. The principle causal agents *M. nivale* and *M. majus* were also detected on average in 96 and 95% of stem bases of English wheat crops respectively. Therefore, BFR and the *Microdochium* spp. are also ubiquitous in English wheat crops. In contrast the *Microdochium* spp. were the least detected species in terms of incidence and biomass in soil samples (chapter 3). These results in chapter 3 suggest the *Microdochium* spp. is a predominately seed borne pathogen. This study is in agreement with Parry *et al*. (1995) who suggested seed borne inoculum is the most important source of inoculum. Sowing date and previous crop were the main agronomy practices influencing BFR and *Microdochium* spp. DNA in soil. Early sowing favoured BFR severity and increased *M. nivale* DNA concentrations in the soil compared to later established crops. The highest *M. nivale* and *M. majus* DNA in stem bases occurred when the previous crop grown was a legume. It could not be stated why this occurred as legumes are not considered a host of the *Microdochium* spp. Despite this, environmental conditions over the autumn/winter period had the greatest, but contrasting, effect on the BFR and the *Microdochium* spp. (chapter 3 and 4). In chapter 3 BFR was negatively correlated with mean atmospheric temperatures but positively with rainfall and humidity. In contrast *M. nivale* and *M. majus* DNA in stem bases was positively correlated with mean atmospheric temperatures and negatively associated with rainfall and humidity. These correlations contradict the findings in chapter 4 that showed *M. nivale* DNA in stem bases was highest in areas with the lowest $Temp_{min}$ range and *M. majus* in the lowest of the $Temp_{max}$ range. Principle component analysis (PCA) also showed weak or no association between BFR and the *Microdochium* spp. However, BFR in this study was affected by foliar fungicides. Crops treated with fungicides that contained prothioconazole at T1 had lower BFR index at GS 37-45 than other treatment combinations. Prothioconazole has been shown to reduce fusarium head blight which is caused by a range of *Fusarium* spp. including *M. nivale* and *M. majus* (Edwards and Godley, 2010). This effect was not shown from seed treatments that contained prothioconazole. It could be that foliar fungicides that contain prothioconazole controlled species that cause BFR later in the season such *F. avenaceum* or *F. culmorum.*

7.2 FUTURE WORK

Several approaches to further the work conducted in this study are proposed:

- Chapter 3 showed AG 2-1 is widespread in English wheat crops. This species is known as a serious pathogen of *Brassica* spp. Further research should investigate control and yield losses from AG 2-1 to OSR as this information is currently not known in the UK. This can be achieved with similar field experiments to chapter 5 that look at the influence of AG 2-1 on OSR from emergence to harvest yield. Sedaxane which had good efficacy to AG 2-1 in chapter 6 should be investigated as a potential control option.

- In chapter 3 the SBD complex pathogens occurred frequently together in the same crops. A controlled environment study could be conducted to investigate the effect of individual species (e.g. *R. cerealis*) compared to a combination of species (e.g. *R. cerealis* + *M. nivale*) on the development of wheat. The aim would be to investigate interactions between species as suggested in chapter 5 between *R. cerealis* and BFR causing species and also to quantify yield losses from individual compared to a combination of species.

- In chapter 3 *Oculimacula* spp. and *R. cerealis* were frequently detected on the roots of wheat. This can be initially investigated be transferring pre-germinated wheat seeds with the radicle and seminal roots present onto tap water agar plates inoculated with *Oculimacula* spp. or *R. cerealis* to observe if any visual lesions on roots develop. If lesions are observed then Koch's postulates should be performed to provide evidence these species are the causative agents of root lesions. This could then lead into future work investigating any effect on crop development.

- Results in chapter 4 suggested OSR in the rotation is selecting for AG 2-1 in soil. This should be investigated in a controlled environment experiment with OSR and wheat grown in different rotational combinations in soil inoculated with AG 2-1. Soil samples can be collected at various times and DNA extracted to quantify changes in AG 2-1 biomass. If OSR is shown to increases AG 2-1 biomass in soil then this experiment could be replicated in field trials to quantify changes in the field environment.

- The seed treatment mixture of sedaxane + fludioxonil significantly reduced BFR in the field experiments at GS 31 and GS 39 (chapter 5). There is no previous published research that has specifically quantified how long sedaxane (or any other fungicide seed treatment) actively protect plants from disease. Plants could be grown from sedaxane treated seed then inoculated at different intervals to investigate the period of time plants are protected. This information could help inform later timings for foliar fungicides.

- Lesions on the stems of wheat caused by *R. cerealis* observed in chapter 3 and 5 will likely interrupt the water and nutrient transport system but this effect has not been quantified. Controlled environment experiments could be conducted to quantify any reductions in water and nutrient uptake in relation to the category of disease (i.e. slight, moderate and severe). Similar experiments could also be conducted for the *Oculimacula* spp. and the *Microdochium* spp.

- Further characterisation work of *R. solani* AG 2-1 is required. Soil isolations should be conducted to isolate AG 2-1 from soil samples collected throughout the UK focusing on fields were wheat and OSR frequently occur in the rotation. These isolate can be characterised using similar experiments conducted on *R. cerealis* isolates in chapter 6. This would inform heterogeneity between AG 2-1 isolates, sensitivity to fungicides and pathogenicity to wheat and OSR.

8. REFFERENCES

ADAMS JR, G. C. & BUTLER, E. E. 1982. A re-interpretation of the sexuality of *Thanatephorus cucumeris* anastomosis group four. Mycologia, 793-800.

AHDB. Not dated(c). HGCA Recommended List® winter wheat 2010/11. Available from: http://cereals.ahdb.org.uk/media/4308/wwrl-10.pdf. Accessed 20st February 2016.

AHDB. Not dated(c). HGCA Recommended List® winter wheat 2010/11. Available from: http://cereals.ahdb.org.uk/media/4299/wwrl-11.pdf. Accessed 20st February 2016.

AHDB. 2014. Oilseed rape guide. Available from: http://cereals.ahdb.org.uk/media/305093/g55-oilseed-rape-guide-jan-2014-update.pdf. Accessed 1st February 2016.

AHDB 2015. Supply and Demand, 25th November 2015. Available from: https://cereals.ahdb.org.uk/media/773842/25-NOV-BALANCE-SHEET-FINAL.pdf. Accessed 17th May 2016.

AHDB. 2015. Wheat growth guide. Available from: http://cereals.ahdb.org.uk/media/185687/g66-wheat-growth-guide.pdf. Accessed 1st January 2016.

AJAYI, O. & BRADLEY, C. I. 2014. Sensitivity of *Rhizoctonia solani* to SDHI and DMI fungicides. Available from: http://www.apsnet.org/meetings/Documents/2014_meeting_abstracts/aps2014abO195.htm. Accessed 25th January 2016.

ALIFERIS, K. A. & JABAJI, S. 2010. Metabolite composition and bioactivity of *Rhizoctonia solani* sclerotial exudates. Journal of Agricultural and Food Chemistry, 58, 7604-7615.

ANDERSON, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. Annual Review of Phytopathology, 20, 329-347.

ANEES, M., TRONSMO, A., EDEL-HERMANN, V., GAUTHERON, N., FALOYA, V. & STEINBERG, C. 2010. Biotic changes in relation to local decrease in soil conduciveness to disease caused by *Rhizoctonia solani*. European Journal of Plant Pathology, 126, 29-41.

AOYAGI, T., KAGEYAMA, K. & HYAKUMACHI, M. 1998. Characterization and survival of *Rhizoctonia solani* AG2-2 LP associated with large patch disease of zoysia grass. Plant Disease, 82, 857-863.

ASCENCION, L. C., LIANG, W.-J. & YEN, T.-B. 2015. Control of *Rhizoctonia solani* damping-off disease after soil amendment with dry tissues of *Brassica* results from increase in Actinomycetes population. Biological Control, 82, 21-30.

BABIKER, E. M., HULBERT, S. H., SCHROEDER, K. L. & PAULITZ, T. C. 2013. Evaluation of *Brassica* species for resistance to *Rhizoctonia solani* and binucleate *Rhizoctonia* (*Ceratobasidum* spp.) under controlled environment conditions. European Journal of Plant Pathology, 136, 763-772.

BAILEY, D., KLECZKOWSKI, A. & GILLIGAN, C. 2004. Epidemiological dynamics and the efficiency of biological control of soil-borne disease during consecutive epidemics in a controlled environment. New Phytologist, 161, 569-575.

BAILEY, D., OTTEN, W. & GILLIGAN, C. A. 2000. Saprotrophic invasion by the soil-borne fungal plant pathogen *Rhizoctonia solani* and percolation thresholds. New Phytologist, 146, 535-544.

BAINS, P., BENNYPAUL, H., LYNCH, D., KAWCHUK, L. & SCHAUPMEYER, C. 2002. Rhizoctonia disease of potatoes (*Rhizoctonia solani*): Fungicidal efficacy and cultivar susceptibility. American Journal of Potato Research, 79, 99-106.

BARTZ, F. E., CUBETA, M. A., TODA, T., NAITO, S. & IVORS, K. L. 2010. An In Planta Method for Assessing the Role of Basidiospores in Rhizoctonia Foliar Disease of Tomato. Plant Disease, 94, 515-520.

BATEMAN, G. & JENKYN, J. 2001. Biology and control of stem-base diseases of cereals in the UK. Pesticide Outlook, 12, 103-106.

BATEMAN, G. L. 1993. Development of disease symptoms and fungal pathogens on shoot bases in continuous winter wheat, and effects of fungicides. Plant Pathology, 42, 595-608.

BATEMAN, G. L., EDWARDS, S. G., MARSHALL, J., MORGAN, L. W., NICHOLSON, P., NUTTALL, M., PARRY, D. W., SCRANCHER, M. & TURNER, A. S. 2000. Effects of cultivar and fungicides on stem-base pathogens, determined by quantitative PCR, and on diseases and yield of wheat. Annals of Applied Biology, 137, 213-221.

BATEMAN, G. L., MURRAY, G., GUTTERIDGE, R. J. & COŞLKUN, H. 1998. Effects of method of straw disposal and depth of cultivation on populations of Fusarium spp. in soil and on brown foot rot in continuous winter wheat. Annals of Applied Biology, 132, 35-47.

BIERMAN, S., FITT, B. D., VAN DEN BOSCH, F., BATEMAN, G. L., JENKYN, J. & WELHAM, S. 2002. Changes in populations of the eyespot fungi *Tapesia yallundae* and *T. acuformis* under different fungicide regimes in successive crops of winter wheat, 1984–2000. Plant Pathology, 51, 191-201.

BOCK, C. H., WAN, A. M. & FITT, B. D. L. 2009. Development of *Oculimacula yallundae* and *O. acuformis* (eyespot) lesions on stems of winter wheat in relation to thermal time in the UK. Plant Pathology, 58, 12-22.

BOINE, B., RENNER, A.-C., ZELLNER, M. & NECHWATAL, J. 2014. Quantitative methods for assessment of the impact of different crops on the inoculum density of *Rhizoctonia solani* AG2-2IIIB in soil. European Journal of Plant Pathology, 140, 745-756.

BOTHA, A., DENMAN, S., LAMPRECHT, S., MAZZOLA, M. & CROUS, P. 2003. Characterisation and pathogenicity of *Rhizoctonia* isolates associated with black root rot of strawberries in the Western Cape Province, South Africa. *Australasian Plant Pathology,* 32**,** 195-201.

BRADY, N. C. & WEIL, R. R. 2008. The nature and properties of soils, Prentice-Hall Inc.

BRIERLEY, J. L., STEWART, J. A. & LEES, A. K. 2009. Quantifying potato pathogen DNA in soil. Applied Soil Ecology, 41, 234-238.

BUDGE, G. E., SHAW, M. W., COLYER, A., PIETRAVALLE, S. & BOONHAM, N. 2009. Molecular tools to investigate *Rhizoctonia solani* distribution in soil. Plant Pathology, 58, 1071-1080.

BURNETT, F., ELLIS, C. B., HUGHES, G., KNIGHT, S. M. & RAY, R. 2012. Forecasting eyespot development and yield losses in winter wheat. Agriculture and Horticulture Developmental Board, Project Report No. 491, AHDB, London, UK.

BURPEE, L., SANDERS, P., COLE JR, H. & SHERWOOD, R. 1980a. Anastomosis groups among isolates of *Ceratobasidium cornigerum* and related fungi. Mycologia**,** 689-701.

BURPEE, L., SANDERS, P., COLE JR, H. & SHERWOOD, R. 1980b. Pathogenicity of *Ceratobasidium cornigerum* and related fungi representing five anastomosis groups. Phytopathology, 70, 843-846.

BURTON, R. J., COLEY-SMITH, J. R., WAREING, P. W. & GLADDERS, P. 1988. *Rhizoctonia oryzae* and *R. solani* associated with barley stunt disease in the United Kingdom. Transactions of the British Mycological Society, 91, 409-417.

CAMPBELL, C. L. & NEHER, D. A. 1994. Estimating Disease Severity and Incidence. Epidemiology and Management of Root Diseases. Berlin, Heidelberg: Springer Berlin Heidelberg.

CAMPION, C., CHATOT, C., PERRATON, B. & ANDRIVON, D. 2003. Anastomosis Groups, Pathogenicity and Sensitivity to Fungicides of *Rhizoctonia solani* Isolates Collected on Potato Crops in France. European Journal of Plant Pathology, 109, 983-992.

CARDOSO, J. & ECHANDI, E. 1987. Biological control of Rhizoctonia root rot of snap bean with binucleate *Rhizoctonia*-like fungi. Plant Disease, 71, 167-170.

CARLING, D., KEBLER, K. & LEINER, R. 1986. Interactions between *Rhizoctonia solani* AG-3 and 27 plant species. Plant disease 70, 577-578.

CARLING, D., ROTHROCK, C., MACNISH, G., SWEETINGHAM, M., BRAINARD, K. & WINTERS, S. 1994. Characterization of anastomosis group 11 (AG-11) of *Rhizoctonia solani*. Phytopathology, 84, 1387-1393.

CARLING, D., POPE, E., BRAINARD, K. & CARTER, D. 1999. Characterization of mycorrhizal isolates of *Rhizoctonia solani* from an orchid, including AG-12, a new anastomosis group. Phytopathology, 89**,** 942-946.

CARLING, D. E. 1996. Grouping in Rhizoctonia solani by hyphal anastomosis interactions. In: SNEH, B., JABAJI-HARE, S., NEATE, S. M. & DIJST, G. (eds.) *Rhizoctonia* species. Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Dordrecht, Netherlands: Kluwer Academic.

CARLING, D. E., BAIRD, R. E., GITAITIS, R. D., BRAINARD, K. A. & KUNINAGA, S. 2002a. Characterization of AG-13, a Newly Reported Anastomosis Group of *Rhizoctonia solani*. Phytopathology, 92, 893-899.

CARLING, D. E., HELM, D. J. & LEINER, R. H. 1990. In Vitro Sensitivity of *Rhizoctonia solani* and Other Multinucleate and Binucleate *Rhizoctonia* to Selected Fungicides. Plant Disease, 74, 860-863.

CARLING, D. E., KUNINAGA, S. & BRAINARD, K. A. 2002b. Hyphal Anastomosis Reactions, rDNA-Internal Transcribed Spacer Sequences, and Virulence Levels Among Subsets of *Rhizoctonia solani* Anastomosis Group-2 (AG-2) and AG-BI. Phytopathology, 92, 43-50.

CARLING, D. E., KUNINAGA, S. & LEINER, R. H. 1988. Relatedness within and among intraspecific groups of *Rhizoctonia solani*: A comparison of grouping by anastomsis and by DNA hybridization. The BAT-SHEVA seminar on host - fungus interaction. Jerusalem, Israel.

CARLING, D. E., LEINER, R. H. & KEBLER, K. M. 1987. Characterization of a New Anastomosis Group (AG-9) of *Rhizoctonia solani*. Phytopathology, 77, 1609-1612.

CASTRO, C., DAVIS, J. & WIESE, M. 1988. Quantitative Estimation of *Rhizoctonia solani* AG-3 in Soil. Phytopathology, 78, 1287-1292.

CECCHINI, G. 2003. Function and Structure of Complex II of the Respiratory Chain. Annual Review of Biochemistry, 72, 77-109.

CHAKRABORTY, S., TIEDEMANN, A. V. & TENG, P. S. 2000. Climate change: potential impact on plant diseases. Environmental Pollution, 108, 317-326.

CHEL, I. & BAKER, R. 1980. Induction of suppressiveness to *Rhizoctonia solani* in soil. Phytopathology, 70, 994-998.

CHEN, H.-G., CAO, Q.-G., XIONG, G.-L., LI, W., ZHANG, A.-X., YU, H.-S. & WANG, J.-S. 2010. Composition of Wheat Rhizosphere Antagonistic Bacteria and Wheat Sharp Eyespot as Affected by Rice Straw Mulching. Pedosphere, 20, 505-514.

CHEN, J., LI, G. H., DU, Z. Y., QUAN, W., ZHANG, H. Y., CHE, M. Z., WANG, Z. & ZHANG, Z. J. 2013. Mapping of QTL conferring resistance to sharp eyespot (*Rhizoctonia cerealis*) in bread wheat at the adult plant growth stage. Theoretical and Applied Genetics, 126, 2865-2878.

CHEN, L., ZHANG, Z., LIANG, H., LIU, H., DU, L., XU, H. & XIN, Z. 2008. Overexpression of TiERF1 enhances resistance to sharp eyespot in transgenic wheat. Journal of Experimental Botany, 59, 4195-4204.

CHEN, Y., YAO, J., YANG, X., ZHANG, A.-F. & GAO, T.-C. 2014. Sensitivity of *Rhizoctonia solani* causing rice sheath blight to fluxapyroxad in China. European Journal of Plant Pathology, 140, 419-428.

CIAMPI, M., MEYER, M., COSTA, M., ZALA, M., MCDONALD, B. & CERESINI, P. 2008. Genetic structure of populations of *Rhizoctonia solani* anastomosis group-1 IA from soybean in Brazil. Phytopathology, 98, 932-941.

CLARKSON, J. D. S. & COOK, R. J. 1983. Effect of sharp eyespot (*Rhizoctonia cerealis)* on yield loss in winter wheat. Plant Pathology, 32, 421-428.

COLBACH, N., DUBY, C., CAVELIER, A. & MEYNARD, J. M. 1997a. Influence of cropping systems on foot and root diseases of winter wheat: fitting of a statistical model. European Journal of Agronomy, 6, 61-77.

COLBACH, N., LUCAS, P., CAVELIER, N. & CAVELIER, A. 1997b. Influence of cropping system on sharp eyespot in winter wheat. Crop Protection, 16, 415-422.

COLBACH, N., MAURIN, N. & HUET, P. 1996. Influence of cropping system on foot rot of winter wheat in France. Crop Protection, 15, 295-305.

COLEY-SMITH, J. & COOKE, R. 1971. Survival and germination of fungal sclerotia. Annual Review of Phytopathology, 9, 65-92.

COOK, R. J. 2001. Management of wheat and barley root diseases in modern farming systems. Australasian Plant Pathology, 30, 119-126.

COOK, R. J. & HAGLUND, W. 1991. Wheat yield depression associated with conservation tillage caused by root pathogens in the soil not phytotoxins from the straw. Soil Biology and Biochemistry, 23, 1125-1132.

COOK, R. J., OWNLEY, B. H., ZHANG, H. & VAKOCH, D. 2000. Influence of paired-row spacing and fertilizer placement on yield and root diseases of direct-seeded wheat. Crop Science, 40, 1079-1087.

COOK, R. J., SCHILLINGER, W. F. & CHRISTENSEN, N. W. 2002. Rhizoctonia root rot and take-all of wheat in diverse direct-seed spring cropping systems. Canadian Journal of Plant Pathology, 24, 349-358.

CROMEY, M., BUTLER, R., BODDINGTON, H. & MOORHEAD, A. 2002. Effects of sharp eyespot on yield of wheat (*Triticum aestivum*) in New Zealand. New Zealand Journal of Crop and Horticultural Science, 30, 9-17.

CROMEY, M. G., PARKES, R. A. & FRASER, P. M. 2006. Factors associated with stem base and root diseases of New Zealand wheat and barley crops. Australasian Plant Pathology, 35, 391-400.

CROUS, P., GROENEWALD, J. Z. & GAMS, W. 2003. Eyespot of Cereals Revisited: ITS phylogeny Reveals New Species Relationships. European Journal of Plant Pathology, 109, 841-850.

CUBETA, M. & VILGALYS, R. 1997. Population biology of the *Rhizoctonia solani* complex. Phytopathology, 87, 480.

CULLEN, D., LEES, A., TOTH, I. & DUNCAN, J. 2001. Conventional PCR and Real-time Quantitative PCR Detection of *Helminthosporium Solani* in Soil and on Potato Tubers. European Journal of Plant Pathology, 107, 387-398.

CURTIS, B. C. (2002) Wheat in the world. In: CURTIS, B. E., RAJARAM, S. and GOMEZ MACPHERSON, H (eds.) Bread Wheat: Improvement and Production. FAO, Rome, Italy.

DAAMEN, R. & STOL, W. 1990. Surveys of cereal diseases and pests in the Netherlands. 2. Stem-base diseases of winter wheat. Netherlands Journal of Plant Pathology, 96, 251-260.

DEFRA (Department of Environment, Food and Rural Affairs). 2015. Farming Statistics Provisional 2015 cereal and oilseed rape production estimates United Kingdom. Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/469400/structure-june-ukcerealoilseed-statsnotice-20oct15.pdf. Accessed, 17th May 2016.

DEMIRCI, E. 1998. *Rhizoctonia* species and anastomosis groups isolated from barley and wheat in Erzurum, Turkey. Plant Pathology, 47, 10-15.

DILLION-WESTON, W. A. R. & GARRETT, S. D. 1943. *Rhizoctonia solani* associated with a root rot of cereals in Norfolk. Annals of Applied Biology, 30, 73.

DJÉBALI, N., ELKAHOUI, S., TAAMALLI, W., HESSINI, K., TARHOUNI, B. & MRABET, M. 2014. Tunisian *Rhizoctonia solani* AG3 strains affect potato shoot macronutrients content, infect faba bean plants and show in vitro resistance to azoxystrobin. Australasian Plant Pathology, 43, 347-358.

DODMAN, R. & FLENTJE, N. 1970. The mechanism and physiology of plant penetration by *Rhizoctonia solani*. In: PARMETER, J. (ed.) Biology and Pathology of Rhizoctonia solani. Berkley, California: University of California press.

DONN, S., ALMARIO, J., MULLER, D., MOËNNE-LOCCOZ, Y., GUPTA, V. V., KIRKEGAARD, J. A. & RICHARDSON, A. E. 2014. Rhizosphere microbial communities associated with Rhizoctonia damage at the field and disease patch scale. Applied Soil Ecology, 78, 37-47.

DOORNIK, A. W. 1981. Comparison of methods for detection of *Rhizoctonia solani* in soil. Netherlands Journal of Plant Pathology, 87, 173-176.

DUFFY, B. 2000. Combination of pencycuron and *Pseudomonas* fluorescens strain 2-79 for integrated control of rhizoctonia root rot and take-all of spring wheat. Crop Protection, 19, 21-25.

DUTZMANN, S. & SUTY-HEINZE, A. 2004. Prothioconazole: a broad spectrum demethylation-inhibitor (DMI) for arable crops. Pflanzenschutz-Nachrichten Bayer, 57, 249-264.

DVORAK, J., DEAL, K. R., LUO, M. C., YOU, F. M., VON BORSTEL, K., DEHGHANI, H. 2012. The origin of spelt and free-threshing hexaploid wheat. Journal of Heredity 103, 426-41.

EDWARDS, S. G. & GODLEY, N. P. 2010. Reduction of Fusarium head blight and deoxynivalenol in wheat with early fungicide applications of prothioconazole. Food Additives and Contaminants, 27, 629-635.

FANG, X., FINNEGAN, P. M. & BARBETTI, M. J. 2013. Wide variation in virulence and genetic diversity of binucleate *Rhizoctonia* isolates associated with root rot of strawberry in Western Australia. *PLoS ONE,* 8**,** e55877. doi:10.1371/journal.pone.0055877.

FELDMAN, M. 1995. Wheats. In: SMARRT, J., SIMMONDS, NW., (eds.). Evolution of Crop Plants. Harlow, UK: Longman Scientific and Technical, 185–192.

FITT, B. D., HUANG, Y.-J., VAN DEN BOSCH, F. & WEST, J. S. 2006. Coexistence of related pathogen species on arable crops in space and time. Phytopathology, 44.

FITT, B. D. L. & BAINBRIDGE, A. 1983. Dispersal of *Pseudocercosporella herpotrichoides* Spores from Infected Wheat Straw. Journal of Phytopathology, 106, 214-225.

FITT, B. D. L., GOULDS, A. & POLLEY, R. W. 1988. Eyespot (*Pseudocercosporella herpotrichoides*) epidemiology in relation to prediction of disease severity and yield loss in winter wheat—a review. Plant Pathology, 37, 311-328.

FLENTJE, N. T., HELENA, M., STRETTON, H. M. & MCKENZIE, A. R. 1970. Mechanisms of variation in *Rhizoctonia solani*. In: PARMETER, J. (ed.) Biology and Pathology of Rhizoctonia solani. Berkley, California: University of California press.

GAMON, A. J., SERRANO, L. & SURFUS, S. J. The photochemical reflectance index: an optical indicator of photosynthetic radiation use efficiency across species, functional types, and nutrient levels. Oecologia, 112, 492-501.

GARCÍA, V. G., ONCO, M. P. & SUSAN, V. R. 2006. Review. Biology and systematics of the form genus *Rhizoctonia*. Spanish Journal of Agricultural Research, 4, 55-79.

GHORBANI, R., WILCOCKSON, S., KOOCHEKI, A. & LEIFERT, C. 2008. Soil management for sustainable crop disease control: a review. Environmental Chemistry Letters, 6, 149-162.

GILL, J., SIVASITHAMPARAM, K. & SMETTEM, K. 2000. Soil types with different texture affects development of Rhizoctonia root rot of wheat seedlings. Plant and Soil, 221, 113-120.

GILL, J., SIVASITHAMPARAM, K. & SMETTEM, K. 2001a. Effect of soil moisture at different temperatures on Rhizoctonia root rot of wheat seedlings. Plant and Soil, 231, 91-96.

GILL, J., SIVASITHAMPARAM, K. & SMETTEM, K. 2001b. Soil moisture affects disease severity and colonisation of wheat roots by *Rhizoctonia solani* AG-8. Soil Biology and Biochemistry, 33, 1363-1370.

GILL, J. S., SIVASITHAMPARAM, K. & SMETTEM, K. R. J. 2002. Size of bare-patches in wheat caused by *Rhizoctonia solani* AG-8 is determined by the established mycelial network at sowing. Soil Biology and Biochemistry, 34, 889-893.

GLYNN, N., RAY, R., EDWARDS, S., HARE, M., PARRY, D., BARNETT, C. & BECK, J. 2007. Quantitative *Fusarium* spp. and *Microdochium* spp. PCR assays to evaluate seed treatments for the control of Fusarium seedling blight of wheat. Journal of Applied Microbiology, 102, 1645-1653.

GLYNN, N. C., HARE, M. C. & EDWARDS, S. G. 2008. Fungicide seed treatment efficacy against *Microdochium nivale* and *M. majus in vitro* and *in vivo.* Pest Management Science, 64, 793-799.

GLYNNE, M. D. 1953. Production of spores by *Cercosporella herpotrichoides*. Transactions of the British Mycological Society, 36, 46-IN3.

GOLL, M. B., SCHADE-SCHÜTZE, A., SWART, G., OOSTENDORP, M., SCHOTT, J. J., JASER, B. & FELSENSTEIN, F. G. 2014. Survey on the prevalence of *Rhizoctonia* spp. in European soils and determination of the baseline sensitivity towards sedaxane. Plant Pathology, 63, 148-154.

GOULDS, A. & FITT, B. D. L. 1990. Effects of fungicide timing on the severity of eyespot on winter wheat or winter barley inoculated with W-type or R-type isolates of *Pseudocercosporella herpotrichoides*. Crop Protection, 9, 265-270.

GOULDS, A. & POLLEY, R. 1990. Assessment of eyespot and other stem base diseases of winter wheat and winter barley. Mycological Research, 94, 819-822.

GRAEFF, S., LINK, J. & CLAUPEIN, W. Identification of powdery mildew (*Erysiphe graminis* sp. *tritici*) and take-all disease (*Gaeumannomyces graminis* sp. *tritici*) in wheat (*Triticum aestivum* L.) by means of leaf reflectance measurements. Central European Journal of Biology, 1, 275-288.

GUGEL, R. K., YITBAREK, S. M., VERMA, P. R., MORRALL, R. A. A. & SADASIVAIAH, R. S. 1987. Etiology of the rhizoctonia root rot complex of canola in the Peace River region of Alberta. Canadian Journal of Plant Pathology, 9, 119-128.

GUO, Y., LI, W., SUN, H., WANG, N., YU, H. & CHEN, H. 2012. Detection and quantification of *Rhizoctonia cerealis* in soil using real-time PCR. Journal of General Plant Pathology, 78, 247-254.

HAIGH, I., JENKINSON, P. & HARE, M. 2009. The effect of a mixture of seed-borne *Microdochium nivale* var. *majus* and *Microdochium nivale* var. *nivale* infection on *Fusarium* seedling blight severity and subsequent stem colonisation and growth of winter wheat in pot experiments. European Journal of Plant Pathology, 124, 65-73.

HAIGH, I. M. & HARE, M. C. 2012. The Effect of Freezing Temperatures on *Microdochium majus* and *M. nivale* Seedling Blight of Winter Wheat. International Journal of Agronomy, vol. 2012, Article ID 359017. doi:10.1155/2012/359017.

HAMADA, M., S, YIN, Y. N. & MA, Z. H. 2012. Detection of *Rhizoctonia cerealis* in wheat tissues by real-time PCR assay. Journal of Plant Pathology, 94, 215-217.

HAMADA, M. S., YIN, Y., CHEN, H. & MA, Z. 2011a. The escalating threat of *Rhizoctonia cerealis*, the causal agent of sharp eyespot in wheat. Pest Management Science, 67, 1411-1419.

HAMADA, M. S., YIN, Y. & MA, Z. 2011b. Sensitivity to iprodione, difenoconazole and fludioxonil of *Rhizoctonia cerealis* isolates collected from wheat in China. Crop Protection, 30, 1028-1033.

HANCOCK, J. F, 2004. Plant evolution and the origin of crop species. CABI Publishing.

HARDWICK, N. V., JONES, D. R. & SLOUGH, J. E. 2001. Factors affecting diseases of winter wheat in England and Wales, 1989–98. Plant Pathology, 50, 650-652.

HARE, M. C., PARRY, D. W. & BAKER, M. D. 1999. The Relationship Between Wheat Seed Weight, Infection by *Fusarium culmorum* or *Microdochium nivale*, Germination and Seedling Disease. European Journal of Plant Pathology, 105, 859-866.

HARRIS, A., SIWEK, K. & WISEMAN, B. 1997. Interactions between damping-off fungi, antagonists and Capsicum seedlings. Applied Soil Ecology, 6, 251-263.

HARRIS, J. & MOEN, R. 1985. Replacement of *Rhizoctonia solani* on wheat seedlings by a succession of root-rot fungi. Transactions of the British Mycological Society, 84, 11-20.

HAWKSWORTH, D. L. 2011. A new dawn for the naming of fungi: impacts of decisions made in Melbourne in July 2011 on the future publication and regulation of fungal names. IMA fungus, 2, 155-162.

HAWN, E. & VANTERPOOL, T. 1953. Preliminary studies on the sexual stage of *Rhizoctonia solani* Kühn. Canadian Journal of Botany, 31, 699-710.

HAYAKAWA, T., TODA, T., PING, Q., MGHALU, J. M., YAGUCHI, S. & HYAKUMACHI, M. 2006. A new subgroup of *Rhizoctonia* AG-D, AG-D III, obtained from Japanese *zoysia* grass exhibiting symptoms of a new disease. Plant Disease, 90, 1389-1394.

HEBERT, P. D. N. & GREGORY, T. R. 2005. The Promise of DNA Barcoding for Taxonomy. Systematic Biology, 54, 852-859.

HERMAN, M. 1992a. The effect of soil moisture on the incidence of *Rhizoctonia cerealis* and microbial antagonism against it. I. Pathogenicity of *Rhizoctonia cerealis* under different temperature and soil moisture conditions. Scientia Agriculturae Bohemoslovaca, 24, 243-254.

HERMAN, M. 1992b. The effect of soil moisture on the incidence of *Rhizoctonia cerealis* and microbial antagonism against it. II. Antagonism of the soil against *Rhizoctonia cerealis* as affected by temperature and soil water content. Scientia Agriculturae Bohemoslovaca, 24, 317-325.

HIDE, G. & FIRMAGER, J. P. 1990. Effects of an isolate of *Rhizoctonia solani* Kühn AG8 from diseased barley on the growth and infection of potatoes (*Solanum tuberosum* L.). Potato Research, 33, 229-234.

HIGGINS, S. & FITT, B. D. 1984. Production and pathogenicity to wheat of *Pseudocercosporella herpotrichoides* conidia. Journal of Phytopathology, 111, 222-231.

HIRSCH, P. R., MAUCHLINE, T. H. & CLARK, I. M. 2010. Culture-independent molecular techniques for soil microbial ecology. Soil Biology and Biochemistry, 42, 878-887.

HUANG, W., LAMB, D. W., NIU, Z., ZHANG, Y., LIU, L. & WANG, J. 2007. Identification of yellow rust in wheat using in-situ spectral reflectance measurements and airborne hyperspectral imaging. Precision Agriculture, 8, 187-197.

HUMPHREYS, J., COOKE, B. & STOREY, T. 1995. Effects of seed-borne Microdochium nivale on establishment and grain yield of winter-sown wheat. Plant Varieties & Seeds, 8, 107-117.

HWANG, S. F., AHMED, H. U., TURNBULL, G. D., GOSSEN, B. D. & STRELKOV, S. E. 2014. The effect of seed size, seed treatment, seeding date and depth on Rhizoctonia seedling blight of canola. Canadian Journal of Plant Science, 94, 311-321.

HYAKUMACHI, M., PRIYATMOJO, A., KUBOTA, M. & FUKUI, H. 2005. New Anastomosis Groups, AG-T and AG-U, of Binucleate *Rhizoctonia* spp. Causing Root and Stem Rot of Cut-Flower and Miniature Roses. Phytopathology, 95, 784-792.

IMATHIU, S., RAY, R., BACK, M., HARE, M. & EDWARDS, S. 2009. *Fusarium langsethiae* pathogenicity and aggressiveness towards oats and wheat in wounded and unwounded in vitro detached leaf assays. European Journal of Plant Pathology, 124, 117-126.

INNOCENTI, G., ROBERTI, R., MONTANARI, M. & ZAKRISSON, E. 2003. Efficacy of microorganisms antagonistic to *Rhizoctonia cerealis* and their cell wall degrading enzymatic activities. Mycological Research, 107, 421-427.

JENKYN, J., CHRISTIAN, D., BACON, E., GUTTERIDGE, R. & TODD, A. 2001. Effects of incorporating different amounts of straw on growth, diseases and yield of consecutive crops of winter wheat grown on contrasting soil types. The Journal of Agricultural Science, 136, 1-14.

JENKYN, J. F., GUTTERIDGE, R. J., BATEMAN, G. L. & JALALUDDIN, M. 2010. Effects of crop debris and cultivations on the development of eyespot of wheat caused by *Oculimacula* spp. Annals of Applied Biology, 156, 387-399.

JOHNK, J. S., JONES, R., SHEW, H. & CARLING, D. 1993. Characterization of populations of *Rhizoctonia solani* AG-3 from potato and tobacco. Phytopathology, 83, 854-858.

JOHNK, J. S. & JONES, R. K. 2001. Differentiation of Three Homogeneous Groups of *Rhizoctonia solani* Anastomosis Group 4 by Analysis of Fatty Acids. Phytopathology, 91, 821-830.

JORDAN, V. W. & HUTCHEON, J. A. 2003. Influence of cultivation practices on arable crop diseases. Soil Tillage in Agroecosystems, 187-206.

JULIAN, M. C., DULLEMANS, A. M., VAN SILFHOUT, C. H. & KEIJER, J. 1997. Nuclear behavior in homokaryotic and heterokaryotic fruiting of *Thanatephorus cucumeris* (*Rhizoctonia solani*) anastomosis group 1, subgroup IC. Mycologia, 361-374.

JULIÁN, M. C., DEBETS, F. & KEIJER, J. 1996. Independence of Sexual and Vegetative Incompatibility Mechanisms. Phytopathology, 86:566-574.

KALBERER, N. & GISI, U. 1997. Effect of soil matric potential on sharp eyespot in germinating wheat following seed treatment. Zeitschrift für Pflanzenernährung und Bodenkunde, 160, 195-199.

KATARIA, H. & VERMA, P. 1992. *Rhizoctonia solani* damping-off and root rot in oilseed rape and canola. Crop Protection, 11, 8-13.

KATARIA, H. R. & GISI, U. 1989. Recovery from soil and sensitivity to fungicides of *Rhizoctonia cerealis* and *R. solani.* Mycological Research, 92, 458-462.

KATARIA, H. R. & GISI, U. 1999. Selectivity of fungicides within the genus *Rhizoctonia*. In: LYR, H., RUSSELL, P. E., DEHNE, H. W. & SISLER, H. D. (eds.) Modern Fungicides and Antifungal Compounds. Andover, UK.

KHANGURA, R. K., BARBETTI, M. J. & SWEETINGHAM, M. W. 1999. Characterization and Pathogenicity of *Rhizoctonia* Species on Canola. Plant Disease, 83, 714-721.

KING, J. E. & GRIFFIN, M. J. 1985. Survey of benomyl resistance in *Pseudocercosporella herpotrichoides* on winter wheat and barley in England and Wales in 1983. Plant Pathology, 34, 272-283.

KLOEPPER, J. W., LEONG, J., TEINTZE, M. & SCHROTH, M. N. 1980. *Pseudomonas* siderophores: a mechanism explaining disease-suppressive soils. Current Microbiology, 4, 317-320.

KO, W.-H. & HORA, F. K. 1971. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology, 61, 707-710.

KÖHL, J., DE HAAS, B., KASTELEIN, P., BURGERS, S. & WAALWIJK, C. 2007. Population dynamics of *Fusarium* spp. and *Microdochium nivale* in crops and crop residues of winter wheat. Phytopathology, 97, 971-978.

KOTILA, J. E. 1929. Study of the biology of a new spore-forming *Rhizoctonia, Corticium praticola.* Phytopathology, 19, 1059-1099.

KUMAR, S., SIVASITHAMPARAM, K., GILL, J. S. & SWEETINGHAM, M. W. 1999. Temperature and water potential effects on growth and pathogenicity of *Rhizoctonia solani* AG-11 to lupin. Canadian Journal of Microbiology, 45, 389-395.

KUMAR, S., SIVASITHAMPARAM, K. & SWEETINGHAM, M. 2002. Prolific production of sclerotia in soil by *Rhizoctonia solani* anastomosis group (AG) 11 pathogenic on lupin. Annals of Applied Biology, 141**,** 11-18.

KUNINAGA, S., NATSUAKI, T., TAKEUCHI, T. & YOKOSAWA, R. 1997. Sequence variation of the rDNA ITS regions within and between anastomosis groups in *Rhizoctonia solani*. Current Genetics, 32, 237-243.

KURAMAE, E. E., BUZETO, A. L., CIAMPI, M. B. & SOUZA, N. L. 2003. Identification of *Rhizoctonia solani* AG 1-IB in lettuce, AG 4 HG-I in tomato and melon, and AG 4 HG-III in broccoli and spinach, in Brazil. European Journal of Plant Pathology, 109**,** 391-395.

LAINSBURY, M. A. 2014. The UK Pesticide Guide 2014, CABI.

LANDIS. Not dated. Soilscapes. Availble from: http://www.landis.org.uk/soilscapes/. Accessed 20th February 2016.

LEES, A. K., CULLEN, D. W., SULLIVAN, L. & NICOLSON, M. J. 2002. Development of conventional and quantitative real-time PCR assays for the detection and identification of *Rhizoctonia solani* AG-3 in potato and soil. Plant Pathology, 51, 293-302.

LEES, A. K., NICHOLSON, P., REZANOOR, H. N. & PARRY, D. W. 1995. Analysis of variation within *Microdochium nivale* from wheat: evidence for a distinct sub-group. Mycological Research, 99, 103-109.

LEMAŃCZYK, G. 2012. Effects of farming system, chemical control, fertilizer and sowing density on sharp eyespot and *Rhizoctonia* spp. in winter wheat. Journal of Plant Protection Research, 52, 381-396.

LEMAŃCZYK, G. & KWAŚNA, H. 2013. Effects of sharp eyespot (*Rhizoctonia cerealis*) on yield and grain quality of winter wheat. European Journal of Plant Pathology, 135, 187-200.

LI, W., SUN, H., DENG, Y., ZHANG, A. & CHEN, H. 2014. The heterogeneity of the rDNA-ITS sequence and its phylogeny in *Rhizoctonia cerealis*, the cause of sharp eyespot in wheat. Current Genetics, 60, 1-9.

LI, Z., ZHOU, M., ZHANG, Z., REN, L., DU, L., ZHANG, B., XU, H. & XIN, Z. 2011. Expression of a radish defensin in transgenic wheat confers increased resistance to *Fusarium graminearum* and *Rhizoctonia cerealis*. Functional and Integrative Genomics, 11, 63-70.

LIPPS, P. E. & HERR, L. J. 1982. Etiology of *Rhizoctonia cerealis* in Sharp eyespot of wheat. Phytopathology, 72, 1574-1577.

LOCKWOOD, J. L. 1986. Soilborne plant pathogens: concepts and connections. Phytopathology, 76, 20-27.

LU, C., SONG, B., ZHANG, H., WANG, Y. & ZHENG, X. 2015. Rapid Diagnosis of Soybean Seedling Blight Caused by *Rhizoctonia solani* and Soybean Charcoal Rot Caused by *Macrophomina phaseolina* Using LAMP Assays. Phytopathology, 105, 1612-1617.

MACNISH, G. 1985. Methods of reducing rhizoctonia patch of cereals in Western Australia. Plant Pathology, 34, 175-181.

MACNISH, G. 1988. Changes in take-all (*Gaeumannomyces graminis* var. *tritici*), Rhizoctonia root rot (*Rhizoctonia solani*) and soil pH in continuous wheat with annual applications of nitrogenous fertiliser in Western Australia. Animal Production Science, 28, 333-341.

MACNISH, G. & NEATE, S. M. 1996. Rhizoctonia bare patch of cereals. Plant Disease, 80, 965-971.

MAHUKU, G. S., HSIANG, T. & YANG, L. 1998. Genetic diversity of *Microdochium nivale* isolates from turfgrass. Mycological Research, 102, 559-567.

MATSUMOTO, T. & HIRANE, S. 1932. Physiology and parasitism of fungi generally referred to as hypochnus *sasakii Shirai*. I. Differentiation of strains by means of hyphal fusion and culture in differential media Journal of the Japanese society of Tropical Agriculture 4, 370-388.

MATUSINSKY, P., MIKOLASOVA, R., SPITZER, T. & KLEM, K. 2008. Colonization of Winter Wheat Stem Bases by Communities of Pathogenic Fungi. Cereal Research Communications, 36, 77-88.

MAVRODI, O. V., WALTER, N., ELATEEK, S., TAYLOR, C. G. & OKUBARA, P. A. 2012. Suppression of *Rhizoctonia* and *Pythium* root rot of wheat by new strains of *Pseudomonas*. Biological Control, 62, 93-102.

MAZZOLA, M., SMILEY, R. W., ROVIRA, A. D. & COOK, R. J. 1996. Characterization of *Rhizoctonia* isolates, disease occurrence and management in cereals. In: SNEH, B., JABAJI-HARE, S., NEATE, S. & DIJST, G. (eds.) *Rhizoctonia* species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Dordrecht, Netherlands: Kluwer Academic.

MAZZOLA, M. 1997. Identification and pathogenicity of *Rhizoctonia* spp. isolated from apple roots and orchard soils. Phytopathology, 87**,** 582-587

MCCARTNEY, H. A. & FITT, B. D. L. 1998. Dispersal of foliar fungal plant pathogens: mechanisms, gradients and spatial patterns. In: JONES, D. G. (ed.) The Epidemiology of Plant Diseases. Dordrecht: Springer Netherlands.

MEYER, L., WEHNER, F., NEL, L. & CARLING, D. 1998. Characterization of the crater disease strain of *Rhizoctonia solani.* Phytopathology, 88**,** 366-371.

MEYER, M. C., BUENO, C. J., DE SOUZA, N. L. & YORINORI, J. T. 2006. Effect of doses of fungicides and plant resistance activators on the control of Rhizoctonia foliar blight of soybean, and on *Rhizoctonia solani* AG1–IA in vitro development. Crop Protection, 25, 848-854.

MGHALU, M. J., TSUJI, T., KUBO, N., KUBOTA, M. & HYAKUMACHI, M. 2007. Selective accumulation of *Trichoderma* species in soils suppressive to radish damping-off disease after repeated inoculations with *Rhizoctonia solani*, binucleate *Rhizoctonia* and *Sclerotium rolfsii*. Journal of General Plant Pathology, 73, 250-259.

MILLAR, C. S. & COLHOUN, J. 1969. Fusarium diseases of cereals: IV. Observations on *Fusarium nivale* on wheat. Transactions of the British Mycological Society, 52, 57-IN5.

MUMFORD, R., BOONHAM, N., TOMLINSON, J. & BARKER, I. 2006. Advances in molecular phytodiagnostics – new solutions for old problems. European Journal of Plant Pathology, 116, 1-19.

MURRAY, G. M. & BRENNAN, J. P. 2009. Estimating disease losses to the Australian wheat industry. Australasian Plant Pathology, 38, 558-570.

MUZHINJI, N., TRUTER, M., WOODHALL, J. W. & VAN DER WAALS, J. E. 2015a. Anastomosis Groups and Pathogenicity of *Rhizoctonia solani* and Binucleate *Rhizoctonia* from Potato in South Africa. Plant Disease, 99, 1790-1802.

MUZHINJI, N., WOODHALL, J., TRUTER, M. & VAN DER WAALS, J. 2015b. First report of *Rhizoctonia solani* AG 4HG-III causing potato stem canker in South Africa. Industrial Crops and Products, 74, 327-335.

NAIDU, R. A., PERRY, E. M., PIERCE, F. J. & MEKURIA, T. 2009. The potential of spectral reflectance technique for the detection of Grapevine leafroll-associated virus-3 in two red-berried wine grape cultivars. Computers and Electronics in Agriculture, 66, 38-45.

NAITO, S. 1996. Basidiospores dispersal and survival. . In: SNEH, B., JABAJI-HARE, S., NEATE, S. & DIJST, G. (eds.) *Rhizoctonia* species. Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Dordrecht, Netherlands: Kluwer Academic.

NAITO, S. 2004. Rhizoctonia diseases: taxonomy and population biology. In: Proceeding of the international seminar on biological control of soilborne plant diseases, Japan-Argentina Joint Study, Buenos Aires, Argentina, 2004. 18-31.

NAITO, S. 2006. Ecological studies on teleomorphic and anamorphic stages in *Rhizoctonia* fungi. Journal of General Plant Pathology, 72, 400-403.

NEATE, S. 1989. A comparison of controlled environment and field trials for detection of resistance in cereal cultivars to root rot caused by *Rhizoctonia solani*. Plant Pathology, 38, 494-501.

NICHOLSON, P. & TURNER, A. 2000. Cereal stem-base disease-a complex issue. In: The BCPC Conference: Pests and diseases, Volume 1. Proceedings of an international conference held at the Brighton Hilton Metropole Hotel, Brighton, UK, 13-16 November 2000., 2000. British Crop Protection Council, 99-106.

NICHOLSON, P., TURNER, A., EDWARDS, S., BATEMAN, G., MORGAN, L., PARRY, D., MARSHALL, J. & NUTTALL, M. 2002. Development of stem-base pathogens on different cultivars of winter wheat determined by quantitative PCR. European Journal of Plant Pathology, 108, 163-177.

NIELSEN, L., JUSTESEN, A., JENSEN, J. & JØRGENSEN, L. 2013. *Microdochium nivale* and *Microdochium majus* in seed samples of Danish small grain cereals. Crop Protection, 43, 192-200.

OERKE, E. C., 2006. Crop losses to pests. Journal of Agricultural Science, 144, 31-43.

OGOSHI, A. 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. Annual Review of Phytopathology, 25, 125-143.

OGOSHI, A. 1996. Introduction - the genus *Rhizoctonia*. In: SNEH, B., JABAJI-HARE, S., NEATE, S. & DIJST, G. (eds.) *Rhizoctonia* species. Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Dordrecht, Netherlands: Kluwer Academic.

OGOSHI, A., COOK, R. J. & BASSETT, E. N. 1990. *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. Phytopathology, 80, 784-788.

OGOSHI, A., ONIKI, M., ARAKI, T. & UNI, T. 1983. Studies on the anastomosis groups of binucleate *Rhizoctonia* and their perfect state. Journal of the Faculty of Agriculture, Hokkaido University, 61, 244-260.

OKUBARA, P. A. & JONES, S. S. 2011. Seedling resistance to *Rhizoctonia* and *Pythium* spp. in wheat chromosome group 4 addition lines from *Thinopyrum* spp. Canadian Journal of Plant Pathology, 33, 416-423.

OKUBARA, P. A., SCHROEDER, K. L., ABATZOGLOU, J. T. & PAULITZ, T. C. 2014. Agroecological Factors Correlated to Soil DNA Concentrations of *Rhizoctonia* in Dryland Wheat Production Zones of Washington State, USA. Phytopathology, 104, 683-691.

OKUBARA, P. A., SCHROEDER, K. L. & PAULITZ, T. C. 2008. Identification and Quantification of *Rhizoctonia solani* and *R. oryzae* Using Real-Time Polymerase Chain Reaction. Phytopathology, 98, 837-847.

OKUBARA, P. A., STEBER, C. M., DEMACON, V. L., WALTER, N. L., PAULITZ, T. C. & KIDWELL, K. K. 2009. Scarlet-Rz1, an EMS-generated hexaploid wheat with tolerance to the soilborne necrotrophic pathogens *Rhizoctonia solani* AG-8 and *R. oryzae*. Theoretical and Applied Genetics, 119, 293-303.

OLAYA, G., ABAWI, G. & BARNARD, J. 1994. Response of *Rhizoctonia solani* and Binucleate *Rhizoctonia* to Five Fungicides and Control of Pocket Rot of Table Beets with Foliar Sprays. Plant Disease, 78, 1033-1037.

ONIKI, M., OGOSHI, A., ARAKI, T., SAKAI, R. & TANAKA, S. 1985. The perfect state of *Rhizoctonia oryzae* and *R. zeae*, and the anastomosis groups of *Waitea circinata.* Transactions of the Mycological Society of Japan, 26, 189-198.

OPHEL-KELLER, K., MCKAY, A., HARTLEY, D., HERDINA & CURRAN, J. 2008. Development of a routine DNA-based testing service for soilborne diseases in Australia. Australasian Plant Pathology, 37, 243-253.

OROS, G., NAÁR, Z. & MAGYAR, D. 2013. Susceptibility of Wheat Varieties to Soil-Borne *Rhizoctonia* Infection. American Journal of Plant sciences, 4, 2240-2258.

OYEDOTUN, K. S. & LEMIRE, B. D. 2004. The quaternary structure of the *Saccharomyces cerevisiae* succinate dehydrogenase homology modeling, cofactor docking, and molecular dynamics simulation studies. Journal of Biological Chemistry, 279, 9424-9431.

PAPAVIZAS, G., ADAMS, P., LUMSDEN, R., LEWIS, J., DOW, R., AYERS, W. & KANTZES, J. G. 1975. Ecology and epidemiology of *Rhizoctonia solani* in field soil. Phytopathology, 65, 871-877.

PAREMETER, J. R., SHERWOOD, R. T. & PLATT, W. D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology, 59, 1270-1278.

PARNELL, S., GILLIGAN, C., LUCAS, J., BOCK, C. & VAN DEN BOSCH, F. 2008. Changes in fungicide sensitivity and relative species abundance in *Oculimacula yallundae* and *O. acuformis* populations (eyespot disease of cereals) in Western Europe. Plant Pathology, 57, 509-517.

PARRY, D. W. 1990. Plant pathology in agriculture, CUP Archive.

PARRY, D. W., REZANOOR, H. N., PETTITT, T. R., HARE, M. C. & NICHOLSON, P. 1995. Analysis of *Microdochium nivale* isolates from wheat in the UK during 1993. Annals of Applied Biology, 126, 449-455.

PASK, A., PIETRAGALLA, J., MULLAN, D. & REYNOLDS, M. 2012. Physiological breeding II: a field guide to wheat phenotyping, CIMMYT.

PATEL, J. S., BRENNAN, M. S., KHAN, A. & ALI, G. S. 2015. Implementation of loop-mediated isothermal amplification methods in lateral flow devices for the detection of *Rhizoctonia solani*. Canadian Journal of Plant Pathology, 37, 118-129.

PAULITZ, T. C. & SCHROEDER, K. L. 2005. A New Method for the Quantification of *Rhizoctonia solani* and *R. oryzae* from Soil. Plant Disease, 89, 767-772.

PAULITZ, T. C., SMILEY, R. W. & COOK, R. J. 2002. Insights into the prevalence and management of soilborne cereal pathogens under direct seeding in the Pacific Northwest, USA. Canadian Journal of Plant Pathology, 24, 416-428.

PETTITT, T., XU, X. & PARRY, D. 2003. Association of *Fusarium* species in the wheat stem rot complex. European Journal of Plant Pathology, 109, 769-774.

PETTITT, T. R., PARRY, D. W. & POLLEY, R. W. 1996. Effect of temperature on the incidence of nodal foot rot symptoms in winter wheat crops in England and Wales caused by *Fusarium culmorum* and *Microdochium nivale*. Agricultural and Forest Meteorology, 79, 233-242.

PITT, D. 1964. Studies on sharp eyespot disease of cereals. Annals of Applied Biology, 54, 77-89.

PITT, D. 1966. Studies on sharp eyespot disease of cereals: Effects of the disease on the wheat host and the incidence of disease in the field. Annals of Applied Biology, 58, 299-308.

POLLEY, R. W. & THOMAS, M. R. 1991. Surveys of diseases of winter wheat in England and Wales, 1976–1988. Annals of Applied Biology, 119, 1-20.

POLLEY, R. W. & TURNER, J. A. 1995. Surveys of stem base diseases and fusarium ear diseases in winter wheat in England, Wales and Scotland, 1989–1990. Annals of Applied Biology, 126, 45-59.

PREW, R. & MCINTOSH, A. 1975. Effects of Benomyl and Other Fungicides on Take-all, Eyespot and Sharp Eyespot Diseases of Winter Wheat. Plant Pathology, 24, 67-71.

PREW, R. D., ASHBY, J. E., BACON, E. T. G., CHRISTIAN, D. G., GUTTERIDGE, R. J., JENKYN, J. F., POWELL, W. & TODD, A. D. 1995. Effects of Incorporating or Burning Straw, and of Different Cultivation Systems, on Winter Wheat Grown on Two Soil Types, 1985–91. The Journal of Agricultural Science, 124, 173-194.

PRIYATMOJO, A., YAMAUCHI, R., KAGEYAMA, K. & HYAKUMACHI, M. 2001. Grouping of Binucleate *Rhizoctonia* Anastomosis Group D (AG-D) Isolates into Subgroups I and II Based on Whole-Cell Fatty Acid Compositions. Journal of Phytopathology, 149, 421-426.

PUMPHREY, F., WILKINS, D., HANE, D. & SMILEY, R. 1987. Influence of tillage and nitrogen fertilizer on Rhizoctonia root rot (bare patch) of winter wheat. Plant Disease, 71, 125-127.

QU, P., SALDAJENO, M. G. B. & HYAKUMACHI, M. 2013. Mechanism of the generation of new somatic compatibility groups within *Thanatephorus cucumeris* (*Rhizoctonia solani*). Microbes and Environments, 28, 325.

RAAIJMAKERS, J. M., PAULITZ, T. C., STEINBERG, C., ALABOUVETTE, C. & MOËNNE-LOCCOZ, Y. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant and Soil, 321, 341-361.

RATTI, C., BUDGE, G., WARD, L., CLOVER, G., RUBIES-AUTONELL, C. & HENRY, C. 2004. Detection and relative quantitation of Soil-borne cereal mosaic virus (SBCMV) and *Polymyxa graminis* in winter wheat using real-time PCR (TaqMan®). Journal of Virological Methods, 122, 95-103.

RAY, R. V., JENKINSON, P. & EDWARDS, S. G. 2004. Effects of fungicides on eyespot, caused predominantly by *Oculimacula acuformis*, and yield of early-drilled winter wheat. Crop Protection, 23, 1199-1207.

ROBERTS, F. A. & SIVASITHAMPARAM, K. 1986. Identity and pathogenicity of *Rhizoctonia* spp. associated with bare patch disease of cereals at a field site in Western Australia. Netherlands Journal of Plant Pathology, 92, 185-195.

ROBERTS, P. J. 1999. *Rhizoctonia*-forming fungi: a taxonomic guide, Kew, UK, Royal Botanic Gardens.

ROGET, D. 1995. Decline in root rot (*Rhizoctonia solani* AG-8) in wheat in a tillage and rotation experiment at Avon, South Australia. Animal Production Science, 35, 1009-1013.

ROGET, D., VENN, N. & ROVIRA, A. 1987. Reduction of Rhizoctonia root rot of direct-drilled wheat by short-term chemical fallow. Animal Production Science, 27, 425-430.

ROTHROCK, C., WINTERS, S., KINNEY, P. & CARLING, D. 1993. Occurrence of Rhizoctonia solani AG-7 in Arkansas. Plant Disease, 77**,** 1262.

ROWE, R. & POWELSON, R. 1973. Epidemiology of *Cercosporella* footrot of wheat: Disease spread. Phytopathology, 63, 984-988.

RUSH, C. M., CARLING, D. E., HARVESON, R. M. & MATHIESON, J. T. 1994. Prevalence and Pathogenicity of Anastomosis Groups of *Rhizoctonia solani* from Wheat and Sugar Beet in Texas. Plant Disease, 78, 349-352.

SALAZAR, O., JULIAN, M. & RUBIO, V. 2000. Primers based on specific rDNA-ITS sequences for PCR detection of *Rhizoctonia solani*, *R. solani* AG 2 subgroups and ecological types, and binucleate *Rhizoctonia*. Mycological Research, 104, 281-285.

SANKARAN, S., MISHRA, A., EHSANI, R. & DAVIS, C. 2010. A review of advanced techniques for detecting plant diseases. Computers and Electronics in Agriculture, 72, 1-13.

SAYLER, R. J. & YANG, Y. 2007. Detection and Quantification of *Rhizoctonia solani* AG-1 IA, the Rice Sheath Blight Pathogen, in Rice Using Real-Time PCR. Plant Disease, 91, 1663-1668.

SCHNEEBELI, K., MATHESIUS, U. & WATT, M. 2015. *Brachypodium distachyon* is a pathosystem model for the study of the wheat disease rhizoctonia root rot. Plant Pathology, 64, 91-100.

SCHNEIDER, J., SCHILDER, M. & DIJST, G. 1997. Characterization of *Rhizoctonia solani* AG 2 isolates causing bare patch in field grown tulips in the Netherlands. European Journal of Plant Pathology, 103, 265-279.

SCHOCH, C. L., SEIFERT, K. A., HUHNDORF, S., ROBERT, V., SPOUGE, J. L., LEVESQUE, C. A., CHEN, W. & CONSORTIUM, F. B. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences, 109, 6241-6246.

SCHROEDER, K. & PAULITZ, T. 2008. Effect of inoculum density and soil tillage on the development and severity of Rhizoctonia root rot. Phytopathology, 98, 304-314.

SCHROEDER, K., SHETTY, K. & PAULITZ, T. 2011. Survey of *Rhizoctonia* spp. from wheat soils in the US and determination of pathogenicity on wheat and barley. Phytopathology, 101.

SCOTT, P. R. 1971. The effect of temperature on eyespot (*Cercosporella herpotrichoides*) in wheat seedlings. Annals of Applied Biology, 68, 169-175.

SCOTT, P. R. & HOLLINS, T. W. 1974. Effects of eyespot on the yield of winter wheat. Annals of Applied Biology, 78, 269-279.

SHARON, M., KUNINAGA, S., HYAKUMACHI, M., NAITO, S. & SNEH, B. 2008. Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. Mycoscience, 49, 93-114.

SHARON, M., KUNINAGA, S., HYAKUMACHI, M. & SNEH, B. 2006. The advancing identification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. Mycoscience, 47, 299-316.

SHEWRY, P. R. 2009. Wheat. Journal of Experimental Botany, 60, (6), 1537–1553.

SLOPEK, S. & LABUN, T. 1990. Incidence of eyespot in barley in Alberta, 1989. Canadian plant disease survey. 70 (1).

SMILEY, R. & UDDIN, W. 1993. Influence of soil temperature on Rhizoctonia root rot (*R. solani* AG-8 and *R. oryzae*) of winter wheat. Phytopathology, 83, 777-785.

SMILEY, R. W., COLLINS, H. P. & RASMUSSEN, P. E. 1996. Diseases of wheat in long-term agronomic experiments at Pendleton, Oregon. Plant Disease, 80, 813-820.

SMILEY, R. W., WILKINS, D. E. & KLEPPER, E. L. 1990. Impact of fungicide seed treatments on Rhizoctonia root rot, take-all, eyespot, and growth of winter wheat. Plant Disease, 74, 782-787.

SMITH, J., KIDWELL, K., EVANS, M., COOK, R. & SMILEY, R. 2003a. Assessment of Spring Wheat Genotypes for Disease Reaction to AG-8 in Controlled Environment and Direct-Seeded Field Evaluations. Crop Science, 43, 694-700.

SMITH, J., KIDWELL, K., EVANS, M., COOK, R. & SMILEY, R. 2003b. Evaluation of Spring Cereal Grains and Wild Germplasm for Resistance to AG-8. Crop Science, 43, 701-709.

SNEH, B., BURPEE, L. & OGOSHI, A. 1998. Identyfication of *Rhizoctonia* species. APS PRESS, The American Phytopath. Society, St. Paul, Minnesota.

SNEH, B., BURPEE, L. L. & OGOSHI, A. 1991. Identification of Rhizoctonia species, St. Paul, Minnesota, APS Press.

SNEH, B., JABAJI-HARE, S., NEATE, S. & DIJST, G. 1996. *Rhizoctonia* species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control.

SPARROW, L. A., RETTKE, M. & CORKREY, S. R. 2015. Eight years of annual monitoring of DNA of soil-borne potato pathogens in farm soils in south eastern Australia. Australasian Plant Pathology, 44, 191-203.

SPURLOCK, T. N., ROTHROCK, C. S. & MONFORT, W. S. 2015. Evaluation of Methods to Quantify Populations of *Rhizoctonia* in Soil. Plant Disease, 99, 836-841.

STALPERS, J. & ANDERSEN, T. 1996. A Synopsis of the Taxonomy of Teleomorphs Connected with *Rhizoctonia* S.L. In: SNEH, B., JABAJI-HARE, S., NEATE, S. & DIJST, G. (eds.) *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Springer Netherlands.

STRANGE, R. N. & SCOTT, P. R. 2005. Plant disease: a threat to global food security. Phytopathology, 43.

STRAUSBAUGH, C. A., BRADLEY, C. A., KOEHN, A. C. & FORSTER, R. L. 2004. Survey of root diseases of wheat and barley in southeastern Idaho. Canadian Journal of Plant Pathology, 26, 167-176.

STRAUSBAUGH, C. A., EUJAYL, I. A., PANELLA, L. W. & HANSON, L. E. 2011. Virulence, distribution and diversity of *Rhizoctonia solani* from sugar beet in Idaho and Oregon. Canadian Journal of Plant Pathology, 33, 210-226.

STURROCK, C. J., WOODHALL, J., BROWN, M., WALKER, C., MOONEY, S. J. & RAY, R. V. 2015. Effects of damping-off caused by *Rhizoctonia solani* anastomosis group 2-1 on roots of wheat and oil seed rape quantified using X-ray Computed Tomography and real-time PCR. Frontiers in Plant Science, 6.

SUMMER, D. R. 1996. Sclerotia formation by *Rhizoctonia* species and their survival, Dordrecht, Netherlands, Kluwer Academic.

SUMNER, D. 1985. Virulence of anastomosis groups of *Rhizoctonia solani* and *Rhizoctonia*-like fungi on selected germ plasm of snap bean, lima bean, and cowpea. Plant Disease, 69, 25-27.

SUN, H., WANG, C., LI, W., ZHANG, A., DENG, Y. & CHEN, H. 2015. Characterization of *Rhizoctonia cerealis* sensitivity to thifluzamide in China. Crop Protection, 69, 65-69.

SWEETINGHAM, M. W., CRUICKSHANK, R. H. & WONG, D. H. 1986. Pectic zymograms and taxonomy and pathogenicity of the *Ceratobasidiaceae*. Transactions of the British Mycological Society, 86, 305-311.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. & KUMAR, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution, 30, 2725-2729.

TEO, B., YITBAREK, S., VERMA, P. & MORRALL, R. 1988. Influence of soil moisture, seeding date, and *Rhizoctonia solani* isolates (AG 2-1 and AG 4) on disease incidence and yield in canola. Canadian Journal of Plant Pathology, 10, 151-158.

TEWOLDEMEDHIN, Y. T., LAMPRECHT, S. C., MCLEOD, A. & MAZZOLA, M. 2006. Characterization of *Rhizoctonia* spp. Recovered from Crop Plants Used in Rotational Cropping Systems in the Western Cape Province of South Africa. Plant Disease, 90, 1399-1406.

TEZARA, W., MITCHELL, V. J., DRISCOLL, S. D. & LAWLOR, D. W. 1999. Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. Nature, 401, 914-917.

THORNTON, O'NEILL, HILTON & GILLIGAN 1999. Detection and recovery of *Rhizoctonia solani* in naturally infested glasshouse soils using a combined baiting, double monoclonal antibody ELISA. Plant Pathology, 48, 627-634.

TODA, T. & HYAKUMACHI, M. 2006. Heterokaryon formation in *Thanatephorus cucumeris* anastomosis group 2-2 IV. Mycologia, 98, 726-736.

TODA, T., HYAKUMACHI, M., SUGA, H., KAGEYAMA, K., TANAKA, A. & TANI, T. 1999. Differentiation of *Rhizoctonia* AG-D Isolates from Turfgrass into Subgroups I and II Based on rDNA and RAPD Analyses. European Journal of Plant Pathology, 105, 835-846.

TODA, T., MUSHIKA, T. & HYAKUMACHI, M. 2004. Development of specific PCR primers for the detection of *Rhizoctonia solani* AG 2-2 LP from the leaf sheaths exhibiting large-patch symptom on zoysia grass. FEMS Microbiology Letters, 232, 67-74.

TOMLINSON, J. A., BOONHAM, N. & DICKINSON, M. 2010. Development and evaluation of a one-hour DNA extraction and loop-mediated isothermal amplification assay for rapid detection of phytoplasmas. Plant Pathology, 59, 465-471.

TRUTER, M. & WEHNER, F. 2004. Anastomosis grouping of *Rhizoctonia solani* associated with black scurf and stem canker of potato in South Africa. Plant Disease, 88, 83-83.

TURNER, O'HARA, REZANOOR, NUTTALL, SMITH & NICHOLSON 1999. Visual disease and PCR assessment of stem base diseases in winter wheat. Plant Pathology, 48, 742-748.

TURNER, A. S., NICHOLSON, P., EDWARDS, S. G., BATEMAN, G. L., MORGAN, L. W., TODD, A. D., PARRY, D. W., MARSHALL, J. & NUTTALL, M. 2001. Evaluation of diagnostic and quantitative PCR for the identification and severity assessment of eyespot and sharp eyespot in winter wheat. Plant Pathology, 50, 463-469.

TURNER, A. S., NICHOLSON, P., EDWARDS, S. G., BATEMAN, G. L., MORGAN, L. W., TODD, A. D., PARRY, D. W., MARSHALL, J. & NUTTALL, M. 2002. Relationship between brown foot rot and DNA of *Microdochium nivale*, determined by quantitative PCR, in stem bases of winter wheat. Plant Pathology, 51, 464-471.

VAN BRUGGEN, A. C. & ARNESON, P. A. 1984. Resistance in *Rhizoctonia solani* to tolclofos-methyl. Netherlands Journal of Plant Pathology, 90, 95-106.

VAN DEN BOOGERT, P. H. J. F. & LUTTIKHOLT, A. J. G. 2004. Compatible Biological and Chemical Control Systems for *Rhizoctonia solani* in Potato. European Journal of Plant Pathology, 110, 111-118.

VAN DER HOEVEN, E. & BOLLEN, G. 1980. Effect of benomyl on soil fungi associated with rye. 1. Effect on the incidence of sharp eyespot caused by *Rhizoctonia cerealis*. Netherlands Journal of Plant Pathology, 86, 163-180.

VERMA, P. R. 1996. Oilseed rape and canola diseases incited by *Rhizoctonia* species. *Rhizoctonia* species: Taxonomy, molecular biology, ecology, pathology and disease control. Springer.

VINCELLI, P. C. & BEAUPRE, C. M.-S. 1989. Comparison of media for isolating *Rhizoctonia solani* from soil. . Plant Disease, 73, 1014-1017.

WALE, S. 2004. Integrating knowledge of soilborne pathogens to minimise disease risk in potato production. Australasian Plant Pathology, 33, 167-172.

WALKER, A.-S., AUCLAIR, C., GREDT, M. & LEROUX, P. 2009. First occurrence of resistance to strobilurin fungicides in *Microdochium nivale* and *Microdochium majus* from French naturally infected wheat grains. Pest Management Science, 65, 906-915.

WALSH, K., KORIMBOCUS, J., BOONHAM, N., JENNINGS, P. & HIMS, M. 2005. Using Real-time PCR to Discriminate and Quantify the Closely Related Wheat Pathogens *Oculimacula yallundae* and *Oculimacula acuformis*. Journal of Phytopathology, 153, 715-721.

WAN, A. M., BOCK, C. H., FITT, B. D. L., HARVEY, J. L. & JENKYN, J. F. 2005. Development of *Oculimacula yallundae* and *O. acuformis* (eyespot) on leaf sheaths of winter wheat in the UK in relation to thermal time. Plant Pathology, 54, 144-155.

WARD, E., FOSTER, S. J., FRAAIJE, B. A. & MCCARTNEY, H. A. 2004. Plant pathogen diagnostics: immunological and nucleic acid-based approaches. Annals of Applied Biology, 145, 1-16.

WELLER, D. M. 2007. *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. Phytopathology, 97, 250-256.

WELLER, D. M., COOK, R. J., MACNISH, G. C., BASSETT, E. N., POWELSON, R. L. & PETERSEN, R. R. 1986. Rhizoctonia root rot of small grains favoured by reduced tillage in the Pacific North west. Plant Disease, 70, 70-73.

WHITE, T. J., BRUNS, T., LEE, S. & TAYLOR, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS, M. A., GELFAND, D. H., SNINSKY, J. J. & WHITE, T. J. (eds.) PCR Protocols: a Guide to Methods and Applications. Academic Press, New York.

WHITNEY, H. & PARMETER JR, J. 1963. Synthesis of heterokaryons in *Rhizoctonia solani* Kühn. Canadian Journal of Botany, 41, 879-886.

WIESE, M. V. 1987. Compendium of wheat diseases 2nd, American Phytopathological Society.

WINDEIS, C. & NABBEN, D. J. 1989. Characterization and pathogenicity of anastomosis groups of *Rhizoctonia solarti* isolated from Beta vulgaris. Phytopathology, 79, 83-88.

WOODHALL, J. & LEES, A. 2004. First report of *Rhizoctonia solani* anastomosis group 5 (AG5) on couch grass in Britain. Plant Pathology, 53**,** 538-538.

WOODHALL, J., ADAMS, I., PETERS, J., HARPER, G. & BOONHAM, N. 2013. A new quantitative real-time PCR assay for *Rhizoctonia solani* AG3-PT and the detection of AGs of *Rhizoctonia solani* associated with potato in soil and tuber samples in Great Britain. European Journal of Plant Pathology, 136, 273-280.

WOODHALL, J., WEBB, K., GILTRAP, P., ADAMS, I., PETERS, J., BUDGE, G. & BOONHAM, N. 2012a. A new large scale soil DNA extraction procedure and real-time PCR assay for the detection of *Sclerotium cepivorum* in soil. European Journal of Plant Pathology, 134, 467-473.

WOODHALL, J. W., LAURENSON, L. & PETERS, J. C. 2012b. First report of *Rhizoctonia solani* anastomosis group 5 (AG5) in wheat in the UK. New Disease Reports, 26, 9.

WOODHALL, J. W., LEES, A. K., EDWARDS, S. G. & JENKINSON, P. 2007. Characterization of *Rhizoctonia solani* from potato in Great Britain. Plant Pathology, 56, 286-295.

XIA, X., ZHOA, M., WANG, H. & H, M. 2012. Ecological fitness of fludioxonil-resistant *Rhizoctonia cerealis* strain and its cross resistance to DMIs and *validamycin*. Journal of Food, Agriculture & Environment, 10, 1530-1533.

XU, X. M., NICHOLSON, P., THOMSETT, M. A., SIMPSON, D., COOKE, B. M., DOOHAN, F. M., BRENNAN, J., MONAGHAN, S., MORETTI, A., MULE, G., HORNOK, L., BEKI, E., TATNELL, J., RITIENI, A. & EDWARDS, S. G. 2008. Relationship between the Fungal Complex Causing Fusarium Head Blight of Wheat and Environmental Conditions. Phytopathology, 98, 69-78.

YANAR, Y., YıLMAZ, G., CESMELI, I. & COSKUN, S. 2005. Characterization of *Rhizoctonia solani* isolates from potatoes in turkey and screening potato cultivars for resistance to AG-3 isolates. Phytoparasitica, 33, 370-376.

YANG, H., TOMMERUP, I., SIVASITHAMPARAM, K. & O'BRIEN, P. 1992. Heterokaryon formation with homokaryons derived from protoplasts of *Rhizoctonia solani* anastomosis group eight. Experimental Mycology, 16, 268-278.

YANG, H. A., SIVASITHAMPARAM, K., ALEMOHAMMAD, J., BARTON, J. E. & O'BRIEN, P. A. 1994. Association of *Rhizoctonia* strains with bare patch disease of wheat in Western Australia. Plant Pathology, 43, 878-884.

YANG, J., KHARBANDA, P. D., WANG, H. & MCANDREW, D. W. 1996. Characterization, virulence, and genetic variation of *Rhizoctonia solani* AG-9 in Alberta. Plant Disease, 80, 513-518.

YITBAREK, S. M., VERMA, P. R. & MORRALL, R. A. A. 1987. Anastomosis groups, pathogenicity, and specificity of *Rhizoctonia solani* isolates from seedling and adult rapeseed/canola plants and soils in Saskatchewan. Canadian Journal of Plant Pathology, 9, 6-13.

YULIANTI, T., SIVASITHAMPARAM, K. & TURNER, D. 2007. Saprophytic and pathogenic behaviour of R. solani AG2-1 (ZG-5) in a soil amended with *Diplotaxis tenuifolia* or *Brassica nigra* manures and incubated at different temperatures and soil water content. Plant and Soil, 294, 277-289.

ZADOKS, J. C., CHANG, T. T. & KONZAK, C. F. 1974. A decimal code for the growth stages of cereals. Weed Research, 14, 415-421.

ZEUN, R., SCALLIET, G. & OOSTENDORP, M. 2013. Biological activity of sedaxane – a novel broad-spectrum fungicide for seed treatment. Pest Management Science, 69, 527-534.

ZHANG, Y., LU, J., WANG, J., ZHOU, M. & CHEN, C. 2015. Baseline sensitivity and resistance risk assessmemt of *Rhizoctonia cerealis* to thifluzamide, a succinate dehydrogenase inhibitor. Pesticide Biochemistry and Physiology, 124, 97-102.

APPENDIX - A

Proceedings Crop Protection in Northern Britain 2014

THE OCCURRENCE AND POPULATION DYNAMICS OF *RHIZOCTONIA SOLANI* IN SOIL OF WINTER WHEAT

M B Brown[1], J Woodhall[2], S J Mooney[1] and R V Ray[1]
[1]*University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD*
[2]*The Food and Environment Research Agency, Sand Hutton, N. Yorkshire, YO41 1LZ*
E-mail: stxmb6@nottingham.ac.uk

Summary: There is limited information on the occurrence of *Rhizoctonia solani* in UK wheat fields. Ninety wheat fields were surveyed over two years with soil samples collected at pre-sowing and GS 21-31. DNA concentrations of *R. solani* from soil were quantified using Real-time PCR assays. The predominant anastomosis group (AG) of *R. solani* was AG 2-1 found pre-sowing and at GS 21-31 in 69% and 59% of fields respectively for both years. The North and West Midlands had significantly higher concentrations of AG 2-1 DNA in soil than the South West. Previous crop had a significant effect on concentration of AG 2-1 DNA in soil at GS 21-31 irrespective of sampling year or region. In soil where oilseed rape was the previous crop, AG 2-1 DNA was significantly higher than in soils where continuous wheat was grown. This is the first survey to show that the predominant AG of *R. solani* in English wheat fields is AG 2-1.

INTRODUCTION

The genus *Rhizoctonia* comprises a ubiquitous group of soil inhabiting fungi causing a range of destructive diseases on many of crop species. The most studied *Rhizoctonia* pathogen is *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*). *Rhizoctonia solani* causes damping-off, root rot and stem rot in many crops, including wheat (Ogoshi *et al.*, 1990).

Isolates of *R. solani* are classified into anastomosis groups (AGs) based on the ability of hyphae to fuse and exchange genetic material (Ogoshi, 1987). To date 13 AGs (1-13) belonging to *R. solani* have been identified with several of these containing subgroups (Carling *et al.*, 2002).

The host range of the various AGs of *R. solani* is considerable and contains many economically important crops including potatoes, sugar beet, oilseed rape and cereals (Ogoshi 1987; Schillinger & Paulitz, 2006; Babiker *et al.,* 2013). This wide host range makes rotation ineffective in controlling *R. solani*. Furthermore, in the absence of a host *R. solani* can survive for long periods of time on soil organic matter (Ogoshi, 1987).

Rhizoctonia solani AGs 2-1, 3, 4, 5, 8 and 11 have been reported as a pathogen of wheat in Australia Turkey and the USA (Ogoshi *et al.,* 1990; MacNish & Neate, 1996; Demirci, 1998). A recent European wide soil survey of arable fields (n = 282) using a soil baiting method, revealed that in the UK (n = 32) the predominant AG of *R. solani* was AG 9 (Goll *et al.*, 2013). In addition AGs 2-1, 3 (TB), 5, 8, and 11 were isolated from UK soils (Goll *et al*., 2013). However, samples were taken prior to planting, only in one season (2009/10) and at one time point from uncultivated fields.

Rhizoctonia solani on wheat in the UK has received little attention and is rarely considered by growers and/or agronomists to cause significant disease on the crop. The aim of this study was to determine the incidence and population dynamics of *R. solani* in English wheat fields using molecular methods in relation to agronomy practices (e.g. cultivation, previous crop).

MATERIALS AND METHODS

Soil and plant sampling

Soil samples were collected from 90 randomly selected winter wheat fields in England during 2011/12 and 2012/13 (Figure 1). In each field 36 points were sampled in a gridline design from the same one hectare area of the field throughout the survey. Soil cores were removed from the top 10-15cm. Soil samples were taken post cultivation, but within two weeks of sowing (pre-sowing), and at growth stage (GS) 21-31 of the wheat crop (Zadoks *et al.*, 1974). Agronomy information was recorded including previous crop, soil texture, cultivation and sowing date.

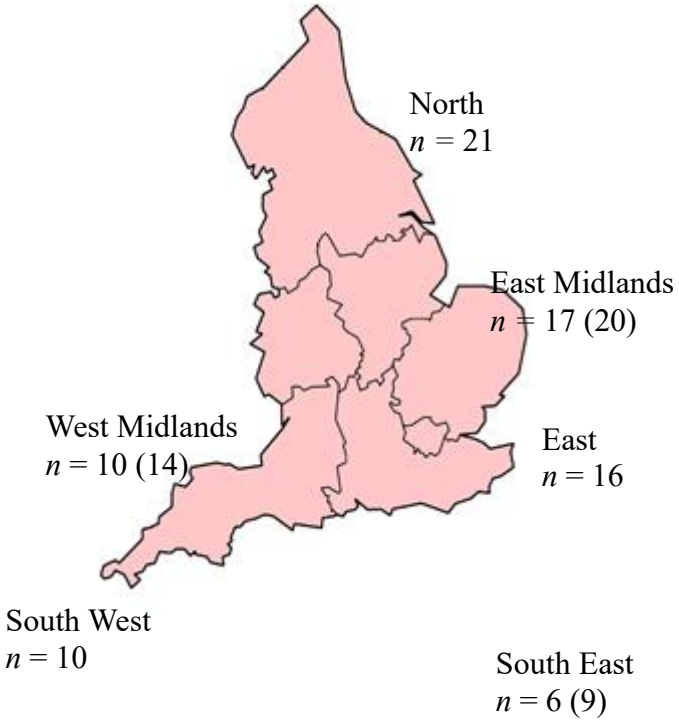


Figure 1. Regional distribution of fields surveyed in England. Pre-sowing *n* = 80 fields and GS 21-31 *n* = 90 fields. Numbers in parentheses are fields sampled at GS-21-31.

Soil DNA extraction

Soil DNA was extracted using the method developed by Woodhall *et al.* (2012). DNA was purified using the Wizard® Magnetic DNA Purification System (Promega UK Ltd) and eluted using a Kingfisher® ML magnetic particle processor (Thermo Electron Corporation).

Quantitative PCR assays

Pathogen DNA was quantified using Real-time PCR (TaqMan®) assays, performed using the Applied Biosystems 7500 real-time PCR system. Targeted *R. solani* AGs were 2-1, 3 (PT), 4 (I, II, II), 5, 8, and 9. Primer and probe sequences are described in Budge *et al*. (2009).

Statistical analysis

All data was analysed using Genstat® 15th edition (VSN International Ltd., UK). DNA data was transformed using log 10 transformation to normalise residuals before analysis using ANOVA unbalanced design.

RESULTS

Incidence of *Rhizoctonia solani* in soil pre-sowing and at GS 21-31 of winter wheat

Rhizoctonia solani AG 2-1 was found pre-sowing and at GS 21-31 in 69% and 59% of fields respectively for both years of sampling (Table 1). There were no significant differences in concentrations of AG 2-1 DNA between years or sampling periods (Table 1). Anastomosis groups 5 and 8 occurred in only 5% of fields sampled at pre-sowing in 2011/12 season (data not shown). AG 3 (PT), 4 and 9 were not detected in soil.

Table 1. Incidence and quantification of AG 2-1 DNA at pre-sowing and GS 21-31. DNA is log10 transformed.

	Pre-sowing*		GS 21-31**		Mean (Year)	
	Incidence (%)	DNA pg g^{-1} of soil	Incidence (%)	DNA pg g^{-1} of soil	Incidence (%)	DNA pg g^{-1} of soil
2011/12	69	0.12 (1.31)	56	-0.05 (0.90)	62	0.03 (1.08)
2012/13	68	-0.08 (0.83)	63	-0.10 (0.8)	66	-0.09 (0.8)
Mean (GS)	69	0.03 (1.07)	59	-0.07 (0.85)		
SED				***P*-value**		
Year	0.390			0.751		
GS	0.388			0.815		
Year.GS	0.552			0.85		

Back-transformed means are in parenthesis

Pre-sowing * 2011/12 42 fields, 2012/13 38 fields

GS21-31 **2011/12 52 fields, 2012/13 38 fields

Effect of agronomy on *R. solani* AG 2-1 DNA in soil pre-sowing and at GS 21-31 of winter wheat

There were no significant interactions between agronomy factors (i.e. previous crop, soil texture, cultivation and sowing date) and year of sampling on concentrations of AG 2-1 DNA in soil quantified at pre-sowing or at GS 21-31 of wheat. Region and previous crop were the only agronomy factors significantly affecting the DNA of AG 2-1 in soil (Figures 2 and 3).

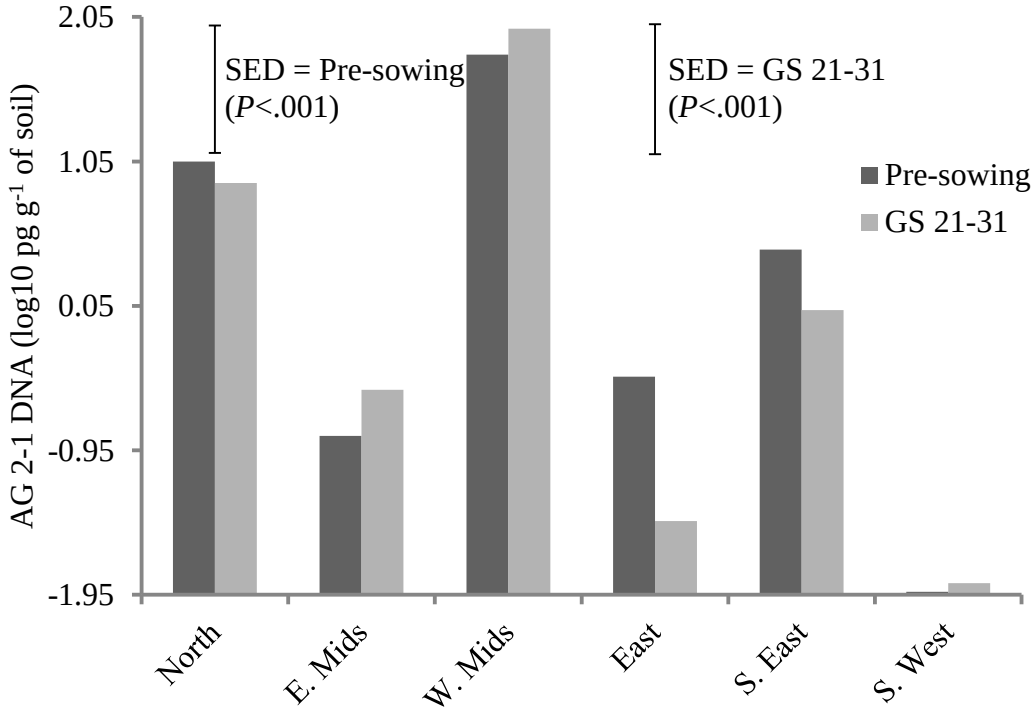


Figure 2. Effect of region on *R. solani* AG 2-1 DNA concentrations at pre-sowing and GS21-31. DNA is log10 transformed.

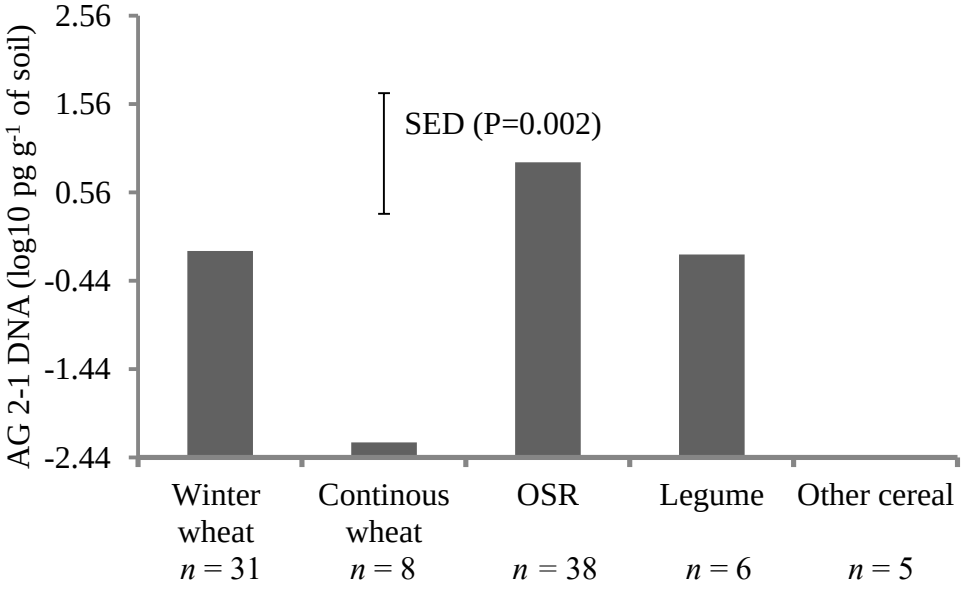


Figure 3. Effect of previous crop on *R. solani* AG 2-1 DNA concentrations at GS21-31. DNA is log10 transformed.

Soils in the West Midlands and the North had significantly higher concentrations of AG 2-1 DNA than soils in the South West region at pre-sowing (P<.001) and at GS 21-31 (P<.001) (Figure 2). Concentrations of AG 2-1 DNA in soil were low on the East side of England, with the South West having the lowest concentrations at both sampling periods.

Previous crop had a significant (P=0.005) effect on concentrations of AG 2-1 in soil at GS 21-31 but not at pre-sowing. When OSR was the previous crop concentrations of AG 2-1 DNA were more than 1000-fold higher than when previous crop was continuous wheat or other cereals (maize and oats) (Figure 3).

DISCUSSION

This survey of English wheat fields showed that the predominant AG of *R. solani* was AG 2-1 found in 69% and 59% of fields at pre-sowing and at GS 21-31 respectively. This is in contrast with the survey carried out by Goll *et al.* (2013), which reported AG 2-1 occurrence in only 13% of locations while the predominant AG was identified as AG 9 occurring in 34% of locations. The difference in results is possibly due to different methods employed to detect *R. solani.* We used AG specific quantitative PCR assays whilst Goll *et al.* (2013) isolated *R. solani* using a soil baiting method which is non-target specific. The low incidence of AG 2-1 occurring in Goll *et al.* (2013) survey suggests soil baiting is not an effective method to detect and isolate AG 2-1. Furthermore, we failed to detect AG 9 in wheat growing fields at pre-sowing (2011/12) and it is possible that differences in geographical locations and/or cropping history of the sampled fields in the individual surveys may be a factor.

Rhizoctonia solani AGs 5 and 8 were detected in only 5% of fields. Both AG 5 and 8 are known pathogens of wheat (MacNish & Neate, 1996; Demirci, 1998; Woodhall *et al.*, 2012). However, their low incidence in soil suggests that the distribution of the two pathogens in England may be restricted to individual fields.

The only two agronomy factors affecting AG 2-1 DNA levels in soil were region and previous crop. The lack of significant interactions between region, previous

crop and year of sampling indicates that the effect of these factors is consistent across different seasons and the effect of previous crop is also similar across regions. Thus, there was a general trend of decline of AG2-1 DNA from the North in a South Eastern direction. It is likely that climatic conditions associated with each region and/or frequency of cropping may help to explain the observed differences.

At GS 21-31 when the previous crop was OSR, concentrations of AG 2-1 DNA were significantly higher than when the previous crop was continuous wheat or other cereals (maize and oats). AG 2-1 is known to be an aggressive pathogen of OSR seedlings causing pre- and post-germination damping-off diseases (Babiker *et al.,* 2013). It is likely that OSR plays an important role in selecting for AG 2-1 in intensive OSR rotations. The increase of pathogen DNA following OSR may be due to volunteer plants or crop residue providing a host for the survival of the pathogen.

This survey has found AG 2-1 to be the predominant *R. solani* in wheat fields in England. However, the final effect on wheat yields by this pathogen is unknown. Further data will be added to this survey to model the risks factors for *R. solani* and additionally for *R. cerealis* in wheat rotations to allow appropriate management strategies to be implemented.

ACKNOWLEDGEMENTS

The authors acknowledge Syngenta for fully funding this project.

REFERENCES

Babiker EM, Hulbert SH, Schroeder KL, Paulitz TC, 2013. Evaluation of *Brassica* species for resistance to *Rhizoctonia solani* and binucleate *Rhizoctonia* (*Ceratobasidum* spp.) under controlled environment conditions. *European Journal of Plant Pathology* 136, (4) 763-72.

Budge GE, Shaw MW, Colyer A, Pietravalle S, Boonham N, 2009. Molecular tools to investigate *Rhizoctonia solani* distribution in soil. Plant Pathology 58, pp. 1071-80.

Carling DE, Baird RE, Gitaitis RD, Brainard KA, Kuninaga S, 2002. Characterisation of AG13, a newly reported anastomosis group of *Rhizoctonia solani*. *Phytopathology* 92, pp. 893-9.

Demirci, E, 1998. *Rhizoctonia* species and anastomosis groups isolated from barley and wheat in Erzurum, Turkey. *Plant Pathology* 47**,** 10-15.

Goll MB, Schade-Schütze A, Swart G, Oostendorp, M, Schott JJ, Jaser, B, Felsenstein FG, 2013. Survey on the prevalence of *Rhizoctonia* spp. in European soils and determination of the baseline sensitivity towards sedaxane. *Plant Pathology* [doi: 10.1111/ppa.12063].

MacNish GC, Neate SM, 1996. Rhizoctonia bare patch of cereals – an Australian perspective. *Plant Disease* 80, pp.965-971.

Ogoshi A, 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. *Annual Review of Phytopathology* 25, pp. 125-43.

Ogoshi A, Cook RJ, Bassett EN, 1990 *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. *Phytopathology* 80, 784-8.

Schillinger WF, Paulitz TC, 2006. Reduction of Rhizoctonia bare patch in wheat with barley rotations. *Plant Disease* 90 (3), 302-6.

Woodhall J W, Webb KM, Giltrap PM, Adams IP, Peters JC, Budge GE, Boonham N, 2012. A new large scale soil DNA extraction procedure and real-time PCR assay for the detection of *Sclerotium cepivorum* in soil. *European Journal of Plant Pathology* 134, (3) 467-73.

Woodhall JW, Laurenson L, Peters JC, 2012. First report of *Rhizoctonia solani* anastomosis group 5 (AG5) in wheat in the UK. *New Disease Report* 26, 9.

Zadoks JC, Chang TT, Konzak, CF, 1974. A decimal code for the growth stages of cereals. *Weed Research* 114 (6), 415-21.

APPENDIX 1

Primer and probes sequences for *Rhizoctonia* spp., *Microdochium* spp. and *Oculimacula* spp. Sequences of primers (F and R affix) and probes (P affix). *Continued*

Target	Primer name	3′ Modification[a]	Sequence (5′– 3′)	Target region	Reference
Rhizoctonia spp.					
AG 2-1	AG 2-1_F		CTT CCT CTT TCA TCC CAC ACA	ITS1	Budge *et al.*
	AG 2-1_R		TGA GTA GAC AGA GGG TCC AAT AAC CTA	ITS1	(2009)
	AG 2-1_P	MGB	AAG TAA ATT CCC CAT CTG T	ITS1	
AG 3-PT	AG3-PT_F		ATG AAG AGT TTG GTT GTA GCT GGT CT	ITS1	Woodhall *et al.*
	AG3-PT_R		TAT TAC AAW*AAA TAA CAA ATA AAT TCC CCA A	ITS1	(2013)
	AG3-PT_P	FAM-TAMRA	CCC TCT TTC ATC CCA CAC ACA CCT G	ITS1	
AG-4HGI	AG-4HGI_F		GGA AGG AAC TTT ATT GGA CCT ACT TCT	ITS1	Woodhall
	AG-4HGI_R		TTA GTA TTA GAT GTG TTA CAT CCA TTA CAT TCA	ITS1	(Pers. Comm)
	AG-4HGI_P	FAM-MGB	GGA CTT CTG TCT ACT TAA TCT	ITS1	
AG-4HGII	AG-4HGII_F		GCA AAG AGG CTG AGG GCT GT	b-tubulin	Budge *et al.*
	AG-4HGII_R		CGG TCT GGG TAC TCT TCA CGA A	b-tubulin	(2009)
	AG-4HGII_P	TAMRA	TAC AGG GCT TCC AGA TTA CCC ACT C	b-tubulin	
AG-4HGIII	AG-4HGIII_F		GTG CAC TTG TGA GAC AGA TTG CT	ITS1	Budge *et al.*
	AG-4HGIII_R		GTT GAA ACT TAG TAT TAG ATG TGT TAC ATC CA	ITS1/5.8S	(2009)
	AG-4HGIII_P	MGB	AAC TTT ATT GGA CCC TTT CT	ITS1	
AG 5	AG-5_F		TGA TCA GGT GCT CGA TGT CGT	b-tubulin	Budge *et al.*
	AG-5_R		CCC TGC AAG CAG TCG GTT	b-tubulin	(2009)
	AG-5_P	MGB	CGC AAA GAG GCC GAG	b-tubulin	

[a] Dual labelled fluorescent probes were labelled with 6-carboxyfluorescein (FAM) 5′modification and either minor groove binding (MGB) or tetra methylrhodamin (TAMRA) 3′modification. [b] Underlined bases denote locked nucleic acid (LNA) bases. *W ambiguous coding for A or T.

Primer and probes sequences for *Rhizoctonia* spp., *Microdochium* spp. and *Oculimacula* spp. Sequences of primers (F and R affix) and probes (P affix).

Target	Primer name	3′ Modification[a]	Sequence (5′– 3′)	Target region	Reference
AG 8	AG-8-F		AGT TGG TTG TAG CTG GTC CAT TAA T	ITS1	Budge *et al.*
	AG-8-R		AGT AGA CAG AGG GGT CCA ATA AAT GA	ITS1	(2009)
	AG-8-P	MGB	TGT GCA CAC CTC CTC	ITS1	
AG 9	AG-9-F		TGC GGA ACT CGT TGA CGC	b-tubulin	Budge *et al.*
	AG-9-R		ACT CGC ATC CCT CGG CTT	b-tubulin	(2009)
	AG-9-P	TAMRA	TTC TCG ACG TTG TGC GTA A	b-tubulin	
R. cerealis	RcF		AAAGCATCGTCGCCATGAG	ITS1	Woodhall *et al.*
	RcR		CTGCCAACACACCGACATGT	ITS1	(submitted)
	RcP	FAM-TAMRA	ATAAAATGGAAGGTAGGTGCGGGTGCATAG	ITS1	
***Microdochium* spp.**					
M. nivale	Mniv1f		TTGGCTTGCACAAACAATACTTTTT	TEF-1α	Nielsen *et al.*
	Mniv1r		AGCACAACAGGCGTGGATAAG	TEF-1α	(2013)
M. majus	Mmajus1f		AACCCCTCCCGGGTCAG	TEF-1α	Nielsen *et al.*
	Mmajus1r		GGATAAACGACACTTGAAGACAGAAAA	TEF-1α	(2013)
***Oculimacula* spp.**					
O. acuformis	CSL TA/F		GCC ACC CTA CTT CGG TAA	ITS1	Walsh *et al.*
	CSLp T/R		ATT CAA GGG TGG AGG TCT GRA C	ITS1	(2005)
	CSL T/Probe	FAM	CTT CTC CGA GAG GCC 5GA CGA CTC TAA	ITS1	
O. yallundae	CSL TY/F		GGG GGC TAC CCT ACT TGG CAG	ITS1	Walsh *et al.*
	CSLp T/R		ATT CAA GGG TGG AGG TCT GRA C	ITS1	(2005)
	CSL T/Probe	FAM	CTT CTC CGA GAG GCC 5GA CGA CTC TAA	ITS1	

[a] Dual labelled fluorescent probes were labelled with 6-carboxyfluorescein (FAM) 5′modification and either minor groove binding (MGB) or tetra methylrhodamin (TAMRA) 3′modification. [b] Underlined bases denote locked nucleic acid (LNA) bases.

Real time PCR assay cycle conditions for *Rhizoctonia*, *Oculimacula* and *Microdochium*-spp

Target	**Cycle conditions**
Rhizoctonia spp.	50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min
Oculimacula spp.	95°C for 3 mins, 40 cycles of 95°C for 30 s and 60°C for 30 s
Microdochium spp.	95 ºC for 2 min 30 s, 35 cycles of 95 ºC for 15 s, 55 ºC for 30 s and 72 ºC for 60 s followed by a final extension at 72 ºC for 7 min.

Real time PCR assay reagents *Rhizoctonia*, *Oculimacula* and *Microdochium*-spp in soil and *planta*

Target	**Sample**	**Master mix (µl)**	**DNA extract (µl)**	**Other reagents**	**F and R-primers (nM)**	**Probe (nM)**	**Primer/probe source**
Rhizoctonia spp.	Soil	TaqMan® Environmental Master Mix 2.0 (12.5 µl)	5	TaqMan® Buffer A, (2.5 µl) Magnesium Chloride ($MgCl_2$), (5.5 µl) Deoxynucleotide Triphosphates (DNTPs) (2 µl)	300	100	MWG Biotech, Germany
	Planta	AmpliTaq Gold® 360 Master Mix (0.125 µl)	2		300	100	MWG Biotech, Germany
Oculimacula spp.	Soil & *Planta*	iQ™ Supermix (20 µl)	5		300	100	Eurofins
Microdochium spp.	Soil & *Planta*	iQ SYBR® Green Supermix (10.5 µl)	2.5		300	100	Eurofins

Appendix 2

Classification of root rot and stem base diseases

Classification of root rot symptoms as described by Strausbaugh *et al.* (2004) -

0 - no lesions evident

1 - lesions on <10% of roots

2 - lesions on 10% - 33% of roots

3 - lesions on 34% - 66% of roots

4 - lesions on >66% of roots

Classification of eyespot, sharp eyespot and BFR severity was based on the key described by Scott and Hollins (1974) –

Clean - No visible lesions on the stem.

Slight - One tiller or the main shoot showing symptoms (lesions girdling less than half the tiller circumference of the plant).

Moderate - More than one tiller or the main shoot of the plant with lesions girdling less than half the tiller circumference or lesions girdling more than half the tiller circumference.

Severe - One tiller or the main shoot of the plant with at least one leaf sheath penetrated or lesions girdling more than half the stem circumference, tissue softened.

Appendix 3

List of farms and counties in which they are located and number of fields soil and plants (wheat) sampled in 2011/12 and 2012/13

Farm	County	No fields sampled 2011/12	2012/13
Vine Farm, Wendy, Nr. Royston, Hertfordshire, SG8 0HJ	Cambridgeshire	2	2
Nr Radbourne Hall, Derbyshire	Derbyshire	2	2
Southburn, Driffield, East Yorkshire, YO25 9ED	East Yorkshire	2	2
The Estate Office, Yanworth, Nr. Cheltenham, GL54 3LQ	Gloucestershire	2	2
Agronomy house, 1 Langley road, higgins lane industrial estate, burscough, lancashire, L40 8JR	Lancashire	2	2
Hall Farm, Cotesbach, Lutterworth, LE17 4HX	Leicestershire	3	3
Burton Fields Farm, Hinckley Road, Burton Hastings, Hinckley, CV11 6RE	Leicestershire	2	2
Haverholme Park Farm, Haverholme, Ewerby, Sleaford, Lincolnshire, NG34 9PF	Lincolnshire	2	2
Eaubrink River Farm, Tilney All Saints, Kings Lynn, Norfolk, PE34 4SQ	Norfolk	3	3
Helming Farm, Thornton Steward, Thorton watlass.	North Yorkshire	2	2
A&E Jones and Son, Baldersby, Thirsk, North Yorkshire	North Yorkshire	3	3
Percy Farms, Ratcheugh Farm, Longhoughton, Alnwick, Northumberland, NE66 3AE	Northumberland	2	2
University of Nottingham, Sutton Bonington, Campus	Nottinghamshire	2	2
Smallstones Farm, Chilson, Charlbury, Chipping Norton/Oxton/Oxford, OX7 3HS	Oxfordshire	3	2
Empingham Rd, Normanton, Oakham, LE15 8RW	Rutland	2	2
Britsh Quinoa company Ltd Sycamore House Hordley, Ellesmere Shropshire	Shropshire	2	2
Home Farm, Chartley, Stafford ST17 0LN	Staffordshire	2	2
Rougham Estates, The Estate Office, Rougham, Bury St Edmunds, Suffolk, IP30 9LZ	Suffolk	5	4
Penn Croft Farms Ltd, The Farm Office, Crondall, Farnham, Surrey, GU10 5PX.	Surrey	2	2
Fitzroy Farming, The Grain Dryer, Nuthurst Lane, Astley, Nuneaton, Warwickshire, CV10 7QH	Warwickshire	2	2
G. Pinder & Son, Pontefract	West Yorkshire	2	2
Berwick Farm, Berwick St Leonard, Salisbury, Wiltshire, SP3 5SN	Wiltshire	3	3
	Total	52	50

Appendix 4

Agronomy factor, number of observations (*n* =) and REML codes for English wheat crops sampled in 2011/12 and 2012/13, number of observations (*n* =) and REML codes

Soil texture	REML code	*n* =	Previous crop	REML code	*n* =
Silty clay loam	1	11	W. wheat	1	33
Sandy clay loam	2	48	Continuous wheat	2	10
Clay/clay loam	3	23	OSR	3	43
Sandy loam	4	14	Legumes (Peas, Beans)	4	6
			Other Cereals (Oats, Maize)	5	10
1st, 2nd or CW			**Cultivation**		
1st wheat	1	59	Minimum tillage	1	57
2nd wheat	2	33	Plough	2	45
CW	3	10			
Sowing date					
1st-14th September	1	18			
15th-30th September	2	22			
1st-14th October	3	40			
15th-31st October	4	15			
November	5	7			

Active ingredients of seed treatments used in English wheat crops sampled in 2011/12 and 2012/13, number of observations (*n* =) and REML codes

Active ingredient	Seed treatment	REML code	*n* =
Prothioconazole + Clothianidin	Redigo - deter	1	43
Prothioconazole + Silthiofam + Clothianidin	Redigo Deter + Latitude	3	12
Fludioxonil + Silthiofam	Beret Gold + Latitude	4	9
Fludioxonil + Difenoconazole	Celest Extra	5	3
Prothioconazole + Fluquinconazole + Clothianidin	Redigo deter + Galmano	5	3
Silthiofam	Latitude	6	5
Prothioconazole	Redigo	7	27

Active ingredients of folair fungiicdes applied at T1 and T2 to English wheat crops sampled in 2011/12 and 2012/13, number of observations (*n* =) and REML codes

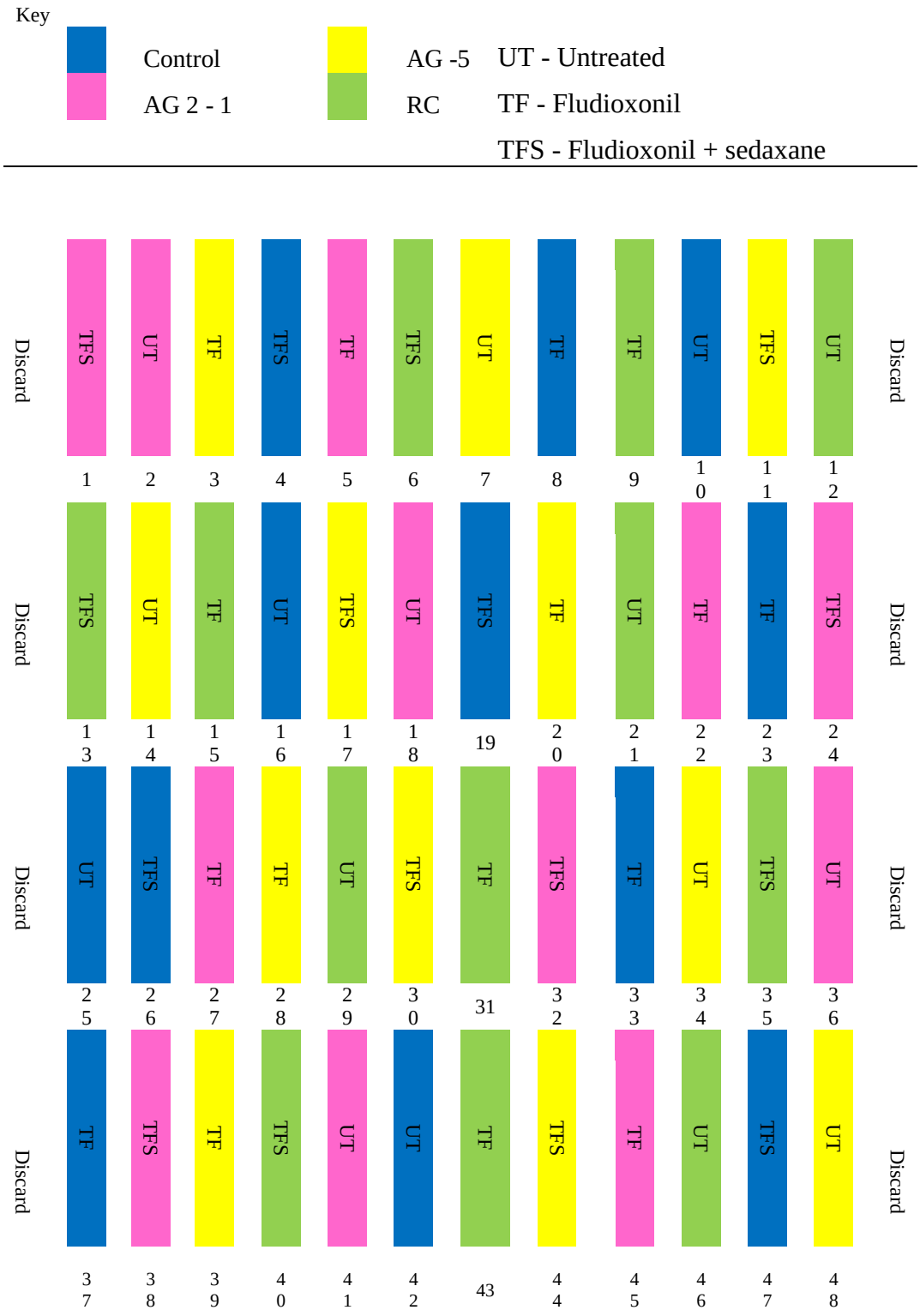
T1 (fungicide)	REML code	*n* =	T2 (fungicide)	REML code	*n* =
MSI+triazole	1	9	Epoxiconazole+strobilurin	1	6
MSI+protioconazole+strobilurin	2	9	Epoxiconazole+strobilurin+SDHI	2	7
SDHI+triazole+other	3	8	Epoxiconazole+triazole+other	3	36
Prothioconazole	4	29	MSI+triazole+other	4	8
Boscalid+other	5	44	Prothioconazole+other	5	35
			Strobilurin+other	6	7

Meteorological factors for English wheat crops sampled in 2011/12 and 2012/13, number of observations (*n* =) and REML codes

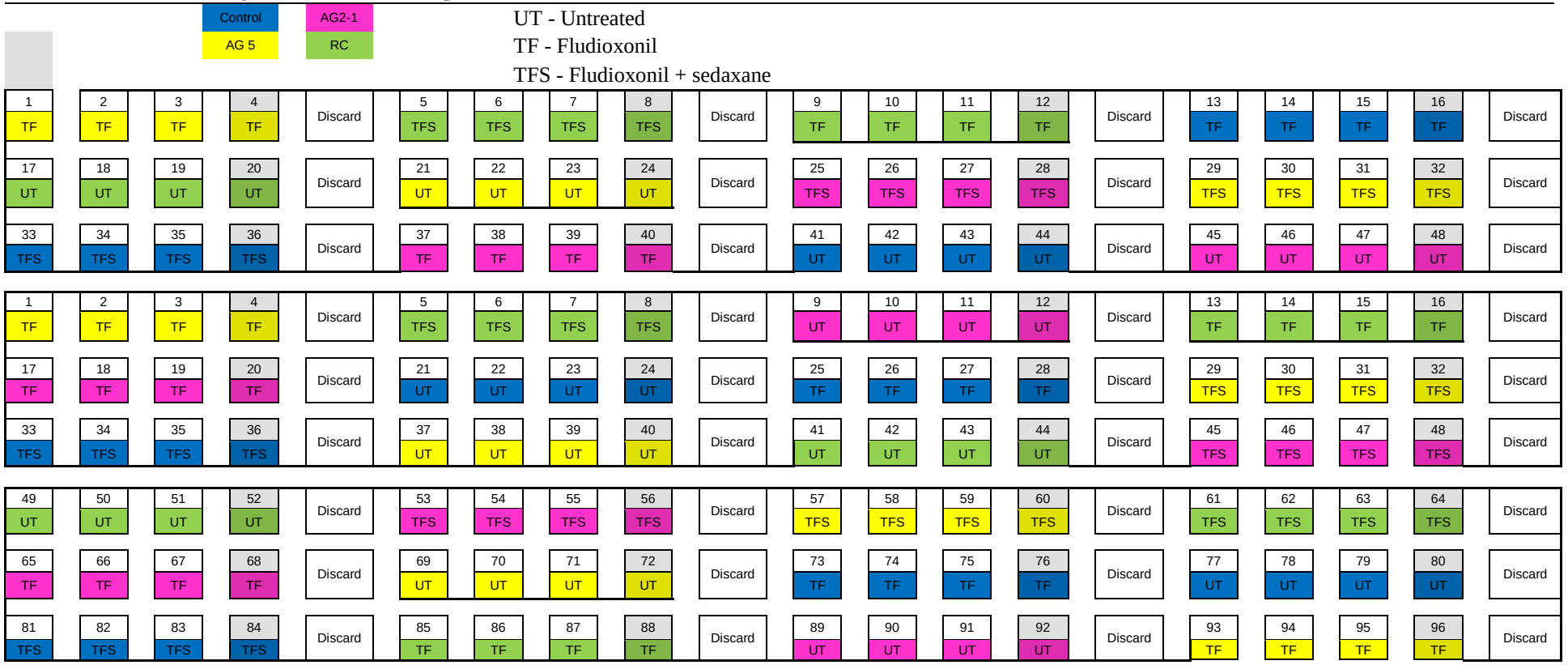
$Temp_{max}$ (°C)	REML code	*n* =	$Temp_{min}$ (°C)	REML code	*n* =	Rainfall (mm)	REML code	*n* =
<8.99	1	32	<2.9	1	9	<199	1	26
9-9.99	2	37	3-3.99	2	41	200-399	2	57
>10	3	33	4-4.99	3	37	>400	3	19
			>5	4	15			

Appendix 5

Randomised block design of 2012/13field experiment



Randomised block design of 2012/13field experiment



Appendix 6

Crop establishment details for field experiments in 2012/13 and 2013/14

	2011/12	**2012/13**
Soil texture	Sandy loam	Sandy loam
Previous crop	Winter oats	Spring OSR
Cultivations	Plough and press (04/09/12) Drilling (04/10/12) Rolling (04//10/12)	Plough and press (30/09/13) Drilling (24/10/13) Rolling (24/10/13)
Drill type	Wintersteiger	Wintersteiger
Plot size	6m x 2m	1m x 1m
Seed density	320 seeds m^2	320 seeds m^2
Seed treatment	Sedaxane (10 g a.i per 100 kg seed) and/or Fludioxonil (5 g a.i per 100 kg seed)	Sedaxane (10 g a.i per 100 kg seed) and/or Fludioxonil (5 g a.i per 100 kg seed)

Fungicide programme for field experiments in 2012/13 and 2013/14

Season	**Date of application**	**Active substance**	**Rate (g ha^{-1})**
2012/13	06/03/2013	Chlorothalonil	500
	01/05/2013	Chlorothalonil	375
		Cyproconazole	50
		Propiconazole	62.5
	16/05/2013	Cyprodinil	75% w/w
	03/06/2013	Epoxiconazole	125
	04/07/2013	Metconazole	60
2013/14	28/03/2014	Chlorothalonil	500
	28/04/2014	Chlorothalonil	375
		Cyproconazole	50
		Propiconazole	62.5
	15/05/2014	Epoxiconazole	50
		Pyraclostrobin	133
	16/05/2014	Cyflufenamid	50
	16/06/2014	Epoxiconazole	37.5
		Metconazole	27.5

Herbicide and insecticide programme for field experiments in 2012/13 and 2013/14

Season	**Date of application**	**Active substance**	**Rate (g ha^{-1})**
2012/13	10/04/2013	Iodosulfuron-methyl-sodium	2
		Mesosulfuron-methyl	10
		Diflufenican	100
		Flufenacet	400
	03/06/2013	Metsulfuron-methyl	200
2013/14	12/03/2014	Iodosulfuron-methyl-sodium	2
		Mesosulfuron-methyl	10
		Mecoprop-P	600
	14/05/2014	Metsulfuron-methyl	111
		Tribenuron-methyl	222
		Zetacypermethrin	100
	16/05/2014	Pinoxaden	100

Appendix 7

Spectral reflectance indices (SRI) for wheat canopy analysis at GS39, GS75 and GS85 where index types are: VI – vegetation index; PI – pigment related index; WI – water index.

Index	Name	Physiological process	Type	Calculation
NDVA	Normalized difference vegetation index	Green area, photosynthetic capacity, N status	VI	[R900-R680]/[R900+R680]
R-NDVI	Red normalized difference vegetation index	Green area, photosynthetic capacity, N status	VI	[R780-R670]/[R780+R670]
G-NDVI	Green normalized difference vegetation index	Green area, photosynthetic capacity, N status	VI	[R780-R550]/[R780+R550]
SRa	Simple ratio	Green biomass	VI	[R800/R680] and [R900/R680]
RARSa	Ratio analysis of reflectance spectra chlorophyll a	Chlorophyll b content	PI	[R675/R700]
RARSc	Ratio analysis of reflectance spectra carotenoid	Carotenoid content	PI	[R760/R500]
PRI	Photochemical reflectance index	Dissipation of excess radiation	PI	[R531-R570]/[R531+R570]
WI	Water index	Plant water status	WI	[R970/R900]
NWI-1	Normalized water index 1	Plant water status	WI	[R970-R900]/[R970+R900]
NWI-2	Normalized water index 2	Plant water status	WI	[R970-R850]/[R970+R850]
NWI-3	Normalized water index 3	Plant water status	WI	[R970-R880]/[R970+R880]
NWI-4	Normalized water index 4	Plant water status	WI	[R970-R920]/[R970+R920]

(Adapted from Pask *et al.*, 2012)

Appendix 8

Above ground dry matter (AGDM) of wheat at GS 13 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*

	GS 39						GS 75					
AGDM (g m2)	**Untreated**		**Fludioxonil**		**Sedaxane + fludioxonil**		**Untreated**		**Fludioxonil**		**Sedaxane + fludioxonil**	
	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14
Control	724	755	689	727	644	764	1566	1681	1511	1578	1574	1584
AG 2-1	580	731	734	717	750	871	1504	1597	1577	1526	1578	1300
AG 5	664	772	746	750	668	792	1488	1604	1639	1575	1460	1778
R. cerealis	636	656	665	661	711	743	1576	1549	1580	1478	1739	1663

Season	2012/13	2013/14					2012/13	2013/14				
	684	745					1566	1576				
			P-value	lsd					P-value	lsd		
Season			0.007	35.9					0.815	40.7		
Inoculation			0.562	38.6					0.488	55.8		
Treatment			0.314	33.4					0.869	48.3		
Inoculation *Treatment			0.491	66.9					0.468	96.7		
Inoculation *season			0.81	49.8					0.209	80.2		
Treatment*Season			0.348	41.4					0.506	69.6		
Inoculation *Treatment*Season			0.976	92.6					0.381	138.4		

Appendix 9

Green area index (GAI) of wheat at GS 13 grown from seed treated with sedaxane and/or fludioxonil or untreated in field plots artificially inoculated with *R. solani* AG 2-1, AG 5 or *R. cerealis*

	GS 39						GS 75					
	Untreated		**Fludioxonil**		**Sedaxane + fludioxonil**		**Untreated**		**Fludioxonil**		**Sedaxane + fludioxonil**	
GAI	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14
Control	3.736	6.045	3.808	5.781	3.783	6.325	4.02	6.15	3.97	6.04	4.52	6.98
AG 2-1	3.881	5.846	4.025	5.942	4.723	6.17	4.73	5.96	3.97	5.89	4	6.02
AG 5	3.763	5.635	4.503	6.635	3.938	6.246	3.78	6.23	4.8	7.06	4.15	7.09
R. cerealis	3.485	5.336	3.646	5.672	4.11	6.405	4.04	7.26	4.17	6.24	4.58	6.69
Season												
2012/13	3.95						4.23					
2013/14	6.003						6.47					

	P-value	lsd	P-value	lsd
Season	<.001	0.139	<.001	0.269
Inoculation	0.537	0.279	0.466	0.283
Treatment	0.141	0.2423	0.587	0.245
Inoculation *Treatment	0.665	0.485	0.357	0.49
Inoculation *season	0.848	0.373	0.391	0.441
Treatment*Season	0.944	0.315	0.829	0.392
Inoculation *Treatment*Season	0.985	0.677	0.889	0.722

Appendix 10

Details of other *Rhizoctonia* spp. used in this study

AG	Nottingham code	Fera code	Syngenta code	Origin	Isolated from:
AG 2-1		1856		Japan	*
AG 2-1	1917		RHI09-159/8	UK	Soil
AG 2-1	1926	2332		UK	Broccoli
AG 2-1	1927	2023		UK	Cauliflower
AG 2-1	1933		RHI09-243/2	UK	Soil
AG 2-1	1934			UK	OSR
AG 2-1	1935		RHI09-213/1	UK	Soil
AG 2-1	1942			UK	OSR
AG 3	1900		RHI09-170/2	UK	Soil
AG 3	1901		RHI09-192/2	UK	Soil
AG 3	1902		RHI09-205/1	UK	Soil
AG 3	1904		RHI09-194/1	UK	Soil
AG 3	1920		RHI09-194/1	UK	Soil
AG 4-II	1834	1834		Japan	Beta vulgaris
AG 4-II	1924			*	*
AG 4-II	1937	2315		UK	Cauliflower
AG 5	1905		RHI09-190/1	UK	Soil
AG 5	1906		RHI09-190/9	UK	Soil
AG 8	1841			Australia	Wheat
AG 8	1907		RH159/1	UK	Soil
AG 8	1928	1911		UK	Soil
AG 9	1908		RHI09-176/8	UK	Soil
AG 9	1909		RHI09-180/2	UK	Soil
AG 9	1911		RHI09-188/11	UK	Soil
AG 9	1912		RHI09-199/2	UK	Soil
AG 9	1913		RHI09-209/1	UK	Soil
AG 9	1914		RHI09-225/2	UK	Soil
AG 11	1915		RHI09-190/2	UK	Soil
Unknown	1739			UK	Wheat

Details of *R. cerealis* isolates used in this study (*continued*)

Nottingham code	Fera code	ITS	County	Farm	Field name	Cultured from:	Date isolated	Growth stage	Visual characteristics
1252	mb1	1	W. Yorkshire	Pontefract	1st ww	stem	2012	GS21-31	Flat
1314	mb2	2	Suffolk	Rougham Estate	Pill box	Stem	2012	GS21-31	Fluffy
1346	mb3	3	N. Yorkshire	Brook house	B-2nd ww	Stem	2012	GS21-31	Flat
1350	mb4	3	N. Yorkshire	Brook house	B-2nd ww	stem	2012	GS21-31	Flat
1368	mb5	4	Shropshire	Jones	2nd ww	Stem	2012	GS21-31	Fluffy
1369	mb6	2	Suffolk	Rougham Estate	Pill box	Stem	2012	GS21-31	Fluffy (not as thick)
1435	mb7	5	Suffolk	Rougham Estate	4 winds	stem	2012	GS21-31	Flat
1446	mb8	9	Surrey	Pen croft	Bartons	Stem	2012	GS21-31	Flat
1448	mb9	5	Suffolk	Rougham Estate	4 winds	stem	2012	GS21-31	Flat
1450	mb10	6	Surrey	Pen croft	Bartons	stem	2012	GS21-31	Flat
1451	mb11	5	Suffolk	Rougham Estate	4 winds	stem	2012	GS21-31	Flat
1480	mb13	5	Surrey	Pen croft	Green park	root	2012	GS21-31	Fluffy
1484	mb14	4	Suffolk	Rougham Estate	4 winds	root	2012	GS21-31	Fluffy
1486	mb15	5	Surrey	Pen croft	Green park	Stem	2012	GS21-31	Flat
1510	mb16	5	N. Yorkshire	Brook house	B 1st ww	Stem	2012	GS21-31	Flat
1514	mb17	7	Surrey	Pen croft	Bartons	Stem	2012	GS21-31	Flat
1556	mb18	8	Wiltshire	Berwick Farm	Venezuela	Stem	2012	GS21-31	Fluffy (not as thick)
1561	mb19	9	Surrey	Pen croft	Bartons	Stem	2012	GS21-31	Fluffy
1584	mb20	10	Lancashire	Ormskirk	2nd ww	stem	2012	GS21-31	Brown hyphae
1585	mb21	10	Lancashire	Ormskirk	2nd ww	stem	2012	GS21-31	Flat (brown)
1595	mb22	2	N. Yorkshire	Brook house	B-1st ww	root	2012	GS21-31	Flat
1605	mb23	2	Staffordshire	Home farm	R. Brown	stem	2012	GS21-31	Fluffy
1726	mb24	9	E. Yorkshire	Pontefract	2nd ww	Stem	2012	GS21-31	Flat
1737	mb25	9	E. Yorkshire	Pontefract	2nd ww	Stem	2012	GS21-31	Flat

Details of *R. cerealis* isolates used in this study

Nottingham code	Fera code	ITS	County	Farm	Field name	Cultured from:	Date isolated	Growth stage	Visual characteristics
1740	mb27	2	Staffordshire	Home farm	R. Brown	Stem	2012	GS21-31	Fluffy
1944	mb28	2	N. Yorkshire	Brook house	50 acre	Stem	2014	GS65	Fluffy (sclerotia)
1947	mb31	11	Shropshire	Harper Adams	Field 2	Stem	2014	GS61	Fluffy
1952	mb36	14	Cornwall	ADAS Field	Sawmills	Stem	2014	GS61	Fluffy
1953	mb37	15	Cornwall	ADAS Field	Sawmills	Stem	2014	GS62	Fluffy
1954	mb38	16	Cornwall	ADAS Field	Sawmills	Stem	2014	GS63	Fluffy
1955	mb39	17	Cornwall	ADAS Field	Sawmills	Stem	2014	GS64	Fluffy
1956	mb40	18	Cornwall	ADAS Field	Sawmills	Stem	2014	GS65	Fluffy
1964	mb48	17	Shropshire	Harper Adams	Field 3	stem	2014	GS61	Fluffy
1973	mb57	13	Cornwall	Adas Field	Sawmills	Stem	2014	GS61	Fluffy
1974	mb58	19	Nottinghamshire	Nottingham University	S24	Stem	2014	GS61	Fluffy
1976	mb60	14	Nottinghamshire	Nottingham University	S24	Stem	2014	GS61	Fluffy
1979	mb65	20	Shropshire	Harper Adams	Field 2	Stem	2014	GS61	Flat
1989	mb75	17	Nottinghamshire	Nottingham Uni	Notts 1	Stem	2014	GS61	Flat (Brown)
1990	mb76	2	Nottinghamshire	Nottingham University	S24	Stem	2014	GS61	Fluffy (sclerotia)
2003	mb89	21	Nottinghamshire	Nottingham University	S24	Stem	2014	GS61	Flat
2014	mb100	23	Nottinghamshire	Nottingham University	Notts 1	Stem	2014	GS61	Flat
2020	mb106	22	Nottinghamshire	Nottingham University	S24	Stem	2014	GS61	Fluffy
2029	mb115	7	Nottinghamshire	Nottingham University	S24	Stem	2014	GS61	Fluffy
2049	mb135	4	Shropshire	Harper Adams	Field 2	Stem	2014	GS61	Fluffy (Buds)
2051	mb137	24	Shropshire	Harper Adams	Field 2	Stem	2014	GS61	Flat fuffy
2053	mb139	18	Shropshire	Harper Adams	Field 1	Stem	2014	GS61	Fluffy
2062	mb148	2	N. Yorkshire	Brook House	50 acre	stem	2014	GS61	Fluffy
2070	mb156	18	Shropshire	Harper Adams	Field 1	Stem	2014	GS61	Fluffy
2076	mb161	12	N. Yorkshire	Brook House	50 acre	stem	2014	GS61	Flat