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1 **Title:** Elite UK winter wheat cultivars differ in their ability to support the colonisation of

2 beneficial root-infecting fungi

3 **Running title:** Wheat root colonisation by beneficial Magnaporthaceae species

4 Sarah-Jane Osborne[1†], Vanessa McMillan[1], Rodger White[2] and Kim E. Hammond-Kosack[1*]

5 [1]Department of Biointeractions and Crop Protection, Rothamsted Research, Harpenden,

6 Hertfordshire, AL5 2JQ, UK

7 [2]Department of Computational and Analytical Systems, Rothamsted Research, Harpenden,

8 Hertfordshire, AL5 2JQ, UK

9 sarah-jane.osborne@ahdb.org.uk, vanessa.mcmillan@rothamsted.ac.uk,

10 rodger.white@rothamsted.ac.uk, kim.hammond-kosack@rothamsted.ac.uk

11 *Corresponding author: kim.hammond-kosack@rothamsted.ac.uk, Tel: +44(0) 1582 938240

12 †Current address: AHDB, Ashton House, Ambury Road South, Huntingdon, Cambridgeshire

13 PE29 3EH

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Highlight

Field based evidence that specific elite UK wheat cultivars can support natural populations of the take-all root disease supressing fungus, *Gaeumannomyces hyphopodioides,* in soil under a first wheat crop.

Abstract

In numerous countries, *Gaeumannomyces* species, within the Magnaporthaceae family, have previously been implicated in the suppression of take-all root disease in wheat. A UK arable isolate collection (n= 47) was gathered and shown to contain *Gaeumannomyces hyphopodioides* and an unnamed Magnaporthaceae species. A novel seedling pot bioassay revealed both species had a similar ability to colonise cereal roots, however rye (*Secale cereale*) was only poorly colonised by the Magnaporthaceae species. To evaluate the ability of 40 elite UK winter wheat cultivars to support soil inoculum of beneficial soil dwelling fungi, two field experiments were carried using a naturally infested arable site in south-east England. The elite cultivars grown in the first wheat situation differed in their ability to support *G. hyphopodioides* inoculum, measured by colonisation on Hereward as a subsequent wheat in a seedling soil core bioassay. In addition, the root colonisation ability of *G. hyphopodioides* was influenced by second wheat cultivar choice. Nine cultivars supported the colonisation of the beneficial root fungus. Our findings provide evidence of complex host genotype-*G. hyphopodioides* interactions occurring under field conditions. This new knowledge could provide an additional soil-based crop genetic management strategy, to help combat take-all root disease.

Key words

Beneficial soil dwelling fungi, biological control of root disease, elite UK wheat cultivars, *Gaeumannomyces hyphopodioides*, Magnaporthaceae family, *Phialophora* species, soil-borne fungi, take-all disease, *Triticum aestivum*, wheat germplasm

Introduction

Take-all is a root disease, caused by the recently reclassified soil-borne ascomycete fungus *Gaeumannomyces tritici* (Walker 1981, Hernández-Restrepo et al. 2016) (previous name *Gaeumannomyces graminis* var. *tritici*), which devastates wheat production worldwide. In a first wheat crop, take-all inoculum will begin to build-up in the soil and then can cause severe disease in second and subsequent wheat crops. The fungus spreads across the root surface by means of runner hyphae. Infection hyphae can subsequently invade the root and destroy the root vascular tissue (Skou 1981), leading to the formation of black necrotic lesions that disrupt water and nutrient uptake (Pillinger et al. 2005). Severe root disease causes several above-ground symptoms including stunted plants, lack of grain formation and premature ripening of the grain, which results in a loss in both grain quality and potential yield.

Historically there has been considerable interest in the biological control of take-all disease using bacterial and fungal species naturally occurring in the soil (reviewed by (Wong 1981, Hornby et al. 1998, Weller et al. 2002, Cook 2003)). However successful biological control under field conditions has often been difficult due to the heterogeneous nature of the soil environment and difficulties in establishing sufficient populations of beneficial microorganisms for consistent and effective control.

Closely related fungal species within the Magnaporthaceae family have previously been implicated in the suppression of take-all disease. For example, *Gaeumannomyces hyphopodioides* (Hernández-Restrepo et al. 2016) (previous names *Phialophora radicicola*, *Phialophora* sp. lobed hyphopodia and *Gaeumannomyces graminis* var. *graminis*) occurs naturally in UK grasslands (Deacon 1973) and is known to suppress take-all disease in wheat in both glasshouse and field experiments (Speakman and Lewis 1978, Martyniuk and Myskow 1984, Wong et al. 1996). Field trials conducted in Poland (Martyniuk and Myskow 1984) and Australia (Wong and Southwell 1980, Wong et al. 1996) examined the effect of artificial inoculation of *G. hyphopodioides* to the soil to protect wheat crops against take-all. However, only varying success was reported. *Gaeumannomyces hyphopodioides* protects wheat roots, against take-all infection, by inducing host resistance (Speakman and Lewis 1978). A related unnamed Magnaporthaceae sp. (Hernández-Restrepo et al. 2016), has previously been isolated from fields in the UK (Ward and Bateman 1999) and in Germany (Ulrich et al. 2000), but it is not known if this species can suppress take-all disease.

76 In this study, we explore the effect of cereal and cultivar genotype on the root colonisation

77 ability of *G. hyphopodioides* and the related Magnaporthaceae species with the aim of

78 understanding whether host genetics can be utilised to support natural populations of these

79 fungal species in field soil.

80 The specific aims of this study were four-fold. Firstly, to develop a new arable derived

81 collection of potentially beneficial fungal root colonisers (*G. hyphopodioides* and related

82 species) and compare this to the existing arable and grassland collection reported by

83 Hernández-Restrepo *et al.* (2016). Secondly, to establish a seedling bioassay with artificial

84 fungal inoculum addition under controlled environment conditions, to explore their root

85 colonisation ability on different cereal species. A range of cereal genotypes were evaluated

86 including oats, rye, triticale and wheat. These were included to compare levels of colonisation

87 found for both the potentially beneficial fungal species and the take-all fungus. Thirdly, to

88 explore whether there were any differences in the ability of current commercial UK winter

89 wheat cultivars to support populations of beneficial root colonisers in a naturally *G.*

90 *hyphopodioides* infested first wheat trial site. To achieve this, a post-harvest soil core

91 bioassay, baited with a single cultivar (Hereward), was used to gauge the amount of infective

92 fungal inoculum. Fourthly, to investigate whether different commercial cultivars varied in

93 their ability to be colonised by *G. hyphopodioides* in the seedling soil core bioassay. Post-

94 harvest soil cores were baited with the same field plot cultivar and compared to the cores

95 baited with Hereward.

96 In the naturally infested *G. hyphopodioides* field site, the results obtained indicate that a

97 series of complex host-microbe interactions exist, but that certain elite wheat genotypes when

98 grown in either a first or second rotational position lead to either medium levels or very low

99 levels of root colonisation by this beneficial species. This provides an important resource for

100 studies into the genetic and mechanistic basis of the interaction as well as potentially

101 providing a novel way of introducing and supporting populations of this fungus under field

102 conditions.

103 **Materials and methods**

104 *Fungal isolations*

105 Isolates of the required species, were gathered post-harvest from three commercial wheat

106 fields and one commercial barley field across the Rothamsted Farm, to establish an isolate

107   collection and for the establishment of the seedling pot bioassay. The field sites had previous

108   histories of natural populations of *G. hyphopodioides* and related species (Supplementary

109   Figure S1). Soil cores were taken (between 50-100 depending on field size) and baited from

110   the four fields as described for the take-all soil core bioassay (McMillan et al. 2011)

111   (Supplementary Table S1). Root pieces with sub-epidermal vesicles resembling previously

112   described *G. hyphopodioides* and related species symptoms were cut as 1 cm long segments

113   and surface sterilised for 5 mins in sodium hypochlorite (1:5 dilution with sterile distilled

114   H_2O), triple rinsed in sterile distilled H_2O, blotted dry on filter paper and plated onto potato

115   dextrose agar (PDA) (Sigma Aldrich®, Dorset, UK) amended with penicillin (50 µg per

116   plate) and streptomycin (50 µg per plate). Plates were incubated at 21°C and cultures

117   resembling *Gaeumannomyces* species were plated onto fresh PDA amended with penicillin

118   and streptomycin and incubated for two weeks. Fungal cultures were then transferred onto

119   fresh PDA plates without antibiotics, incubated until plates were confluent and then stored at

120   4°C. Long-term storage of cultures were maintained as agar plugs in sterile distilled water as

121   described previously (Boesewinkel 1976).

122   *Species identification*

123   To confirm species identity, internal transcribed spacers (ITS) sequencing was carried out.

124   DNA was extracted from freeze-dried fungal mycelium using the protocol from Ward et al.

125   (2005) (modified from Fraaije et al. (1999)). PCR was done to amplify the ITS regions using

126   primers ITS5 (GGAAGTAAAAGTCGTAACAAGG) and ITS4

127   (TCCTCCGCTTATTGATATGC) (White et al. 1990). Each 20 µl reaction contained 10 µl of

128   *Taq* polymerase (REDTaq® ReadyMix™ PCR Reaction Mix, Sigma-Aldrich), 1 µl of each

129   primer (10 µM), 6 µl of sterile distilled H_2O and 2 µl of template DNA (100 ng/µl).PCR

130   conditions were: 95°C 5 min, 30 cycles of 95°C 30 secs, 55°C 1 min, 72°C 1 min and

131   extension at 72°C for 10 min. PCR products were purified using a Qiagen QIAquick PCR

132   Purification Kit, sequenced and identification confirmed using the BLAST tool and searching

133   the NCBI database.

134   *Seedling pot bioassay with artificial inoculum addition*

135   A seedling pot bioassay was designed to evaluate the susceptibility of cereal genotypes to one

136   representative isolate of *G. hyphopodioides* (N.14.13) and the unnamed Magnaporthaceae

137   species (S.09.13) from the culture collection. A range of cereal genotypes were evaluated

138   including those used as controls in the take-all seedling pot bioassay: oats (cv. Gerald,

139   resistant to take-all), rye (cv. Carotop, highly resistant to take-all), triticale (cv. Trilogie,

140   moderately resistant to take-all) and hexaploid wheat (cv. Hereward, highly susceptible to

141   take-all) (McMillan et al. 2014). Additional hexaploid wheat genotypes were the spring

142   wheat commercial cultivar Paragon and Watkins landrace line 1190777; Paragon is

143   susceptible to take-all whilst Watkins line 1190777 is partially resistant to take-all

144   (McMillan, unpublished data), and the *Triticum monococcum* genotypes MDR037

145   (susceptible to take-all) and MDR046 (moderately resistant to take-all) (McMillan et al.

146   2014). Hereward was also used as a negative control in both pot bioassays with non-

147   inoculated potato dextrose agar (PDA).

148   A randomised block design was calculated in GenStat (VSNI, Hemel Hempstead, UK)

149   (Payne et al. 2009) and included three inoculated replicates for each treatment. Soil (type:

150   typical Batcombe) was collected in September 2013 from Great Harpenden I field (after oats)

151   on the Rothamsted Farm, crumbled and mixed and stored at room temperature before use in

152   the seedling pot bioassay. Plastic drinking cups (7.5 cm diameter x 11 cm tall, drilled with 4

153   drainage holes, 3 mm diameter) were filled with a 50 cm³ layer of damp coarse sand and then

154   a 150 g layer of soil. PDA plate inoculum was prepared by macerating 1/6th of a confluent

155   PDA plate of either *G. hyphopodioides* or the Magnaporthaceae sp. with soil, equating to a ~

156   25 g layer. The negative control pots were prepared by macerating 1/6th of a non-colonised

157   PDA plate with soil. A further 50 g of soil was added on top. The soil was lightly watered

158   and ten seeds of each cultivar were placed on the soil surface. Seeds were covered with ~ 2

159   cm layer of horticultural grit and cores were placed in a controlled environment room (16

160   hour day, 15°C day/10°C night, twice weekly watering) for five weeks. After five weeks the

161   roots were washed free of soil and immersed in a white dish to visually examine the roots for

162   colonisation by examining for the presence of sub-epidermal vesicles. The total number of

163   plants and roots and the number of colonised plants and roots were recorded to calculate the

164   percentages of plants and roots infected.

165   *Field trials*

166   Two field trials, to evaluate the ability of elite UK winter wheat cultivars to support natural

167   populations of the *G. hyphopodioides* fungus under a first wheat crop, were established in

168   autumn 2014 and 2015. The two small plot field trials were established in two different parts

169   of the same field, known to have underlying natural populations of *G. hyphopodioides*, on the

170   Rothamsted Farm (Hertfordshire, UK) (Supplementary Table S2). The soil is flinty clay loam

171 soil of the typical Batcombe soil series. The experimental field trials consisted of randomised

172 block designs of five replicates of 40 elite wheat cultivars. The elite wheat cultivars consisted

173 of 36 winter wheat cultivars on the Agriculture and Horticulture Development Board

174 (AHDB) 2013/2014 Recommended List (RL) and two winter wheat cultivars (Evolution and

175 Zulu) on the AHDB 2014/2015 RL. In addition two control cultivars were included, the

176 spring wheat cultivar Cadenza and the winter wheat cultivar Hereward, both with known

177 take-all inoculum building phenotypes (low and high, respectively) (McMillan et al. 2011).

178 The two field trials were grown as first wheat crops, after a one year break crop of winter

179 oilseed rape (2014), the second after winter oilseed rape and then spring oats (2015).

180 Fertilisers, pesticides and growth regulators were applied according to the standard practice

181 of the Rothamsted Farm (Supplementary Table S3).

Soil core bioassay to gauge the amount of fungal inoculum under the first wheat crop

183 Post-harvest soil cores were taken from each plot to set up a soil core bioassay (McMillan et

184 al. 2011) to gauge the infectivity of *G. hyphopodioides* fungal inoculum in the soil under the

185 different elite wheat cultivars. The method involved baiting soil cores with wheat seedlings

186 and fungal colonisation was then assessed visually after five weeks growth in the controlled

187 environment room. The baited wheat seedlings effectively represent a subsequent second

188 wheat crop. Six soil cores (5.5 cm diameter by 10 cm deep) were taken post-harvest in a zig-

189 zag transect from different rows across individual plots using a soil auger. Three of the soil

190 cores were watered and ten seeds of the winter wheat cultivar Hereward (RAGT, Cambridge,

191 UK) were placed on the surface of each of the cores to gauge the amount of infective fungal

192 inoculum after growth of current commercial cultivars. Ten seeds of the field plot cultivar

193 were placed on the surface of each of the three remaining soil cores to test for the possibility

194 of wheat genotype-fungal colonisation interactions. After five weeks growth, the plant roots

195 were washed free of soil and immersed in water in a white dish to visually examine the roots

196 for *G. hyphopodioides* colonisation. Any *G. tritici* lesions were also recorded to identify

197 whether take-all fungal inoculum could build-up in a field with underlying *G.*

198 *hyphopodioides* populations. The percentage of colonised roots were calculated for the two

199 baiting methods and to gauge the amount of *G. hyphopodioides* or *G. tritici* inoculum that

200 were supported under each wheat cultivar for the Hereward baiting. Cultures were isolated

201 from colonised root tissue from soil core bioassay seedling plants, as detailed in the previous

202   pot bioassay fungal isolation methodology, to confirm visual assessments that *G.*

203   *hyphopodioides* was the species present.

204   *Statistical analyses*

205   The colonisation percentages were always transformed using the logit transformation to

206   ensure equal variance. The transformed data from the pot bioassay with different cereal

207   genotypes was then statistically analysed using analysis of variance (ANOVA) in GenStat

208   (VSN International Ltd., Hemel Hempstead, UK).

209   For the field data, a Residual Maximum Likelihood (REML) variance components analysis

210   was used to incorporate the sub-blocking structure within the field trials and auto-regressive

211   models were used when required for spatial adjustment of the field trials to account for the

212   degree of patchiness of fungal inoculum in both the y axis and the x axis across the trial sites.

213   Yield data from the two field trials was also statistically analysed using a REML variance

214   components analysis. A combined REML variance components analysis was then used to

215   pool and analyse data from across the two field seasons together. The *P* value threshold was

216   set at ≤ 0.05 for all tests.

217   *Microscopy analysis*

218   A LEICA M205 FA stereomicroscope and associated LAS-AF6000 software (Leica

219   Microsystems Ltd., UK) were used for all microscopic visualisation and image capture of

220   fungi in the colonised roots. Seedling roots were submerged in water in a petri dish and

221   visualised under the stereomicroscope. Scale bars were generated by the LAS-AF6000

222   software.

223   *Phylogenetic analysis*

224   The 47 *G. hyphopodioides* ITS5-ITS4 rDNA regions, from the pot bioassay and two field

225   trials, were compared to ITS rDNA regions of the top three BLAST hits from the NCBI

226   database for all isolates as well as a subset of *G. graminis, G. hyphopodioides*, *G. tritici*, and

227   the unnamed Magnaporthaceae sp. isolates (Hernández-Restrepo et al. 2016). All ITS5-ITS4

228   rDNA regions for all species were aligned in the software package Geneious (Biomatters Ltd.

229   v8.1.3) and a 498 base pair (bp) region was extracted. A phylogenetic tree was constructed on

230   the 498 bp region using the genetic distance model of Tamura-Nei, the tree build method of

231   neighbour-joining with 1000 bootstrap replicates and a support threshold set at 75% in

232   Geneious. The phylogenetic tree was rooted with the *Pyricularia grisea* strains BR0029 and

233   CR0024. Accession numbers for sequences obtained from the NCBI database can be found in

234   Supplementary Table S4.

235   **Results**

236   *Fungal isolations and phylogenetic analysis*

237   An isolate collection was gathered from soil taken post-harvest from four commercial cereal

238   crops harvested in 2013. The field sites chosen had previously shown some suppression of

239   take-all disease in field experiments carried out between 2009-2012 (Supplementary Figure

240   S1). *Gaeumannomyces hyphopodioides*, the unnamed Magnaporthaceae sp. and other closely

241   related fungal root colonisers in the same family produce sub-epidermal vesicles within the

242   root cortex (Deacon 1974). All of the sampled field sites showed this root colonisation

243   phenotype with between 18 – 82% cores displaying characteristic symptoms for each field

244   (Supplementary Table S1). In total, nine isolates that had formed sub-epidermal vesicle

245   formation in the correct size range, were recovered from the wheat seedlings for further

246   analysis from three sites (Table 1). DNA sequences for the ITS5-ITS4 region were obtained

247   and eight isolates from the collection (excluding isolate S.09.13), showed 99%-100% species

248   identity with *G. hyphopodioides* (NCBI Taxonomy ID: 1940676) strain CPC 26267, *G.*

249   *hyphopodioides* strain CPC 26249 and *G. hyphopodioides* strain CPC 26248 (Hernández-

250   Restrepo et al. 2016), the top three hits for all isolates from the NCBI database (Table 1 and

251   Supplementary Table S4). The ITS5-ITS4 rDNA sequence for the S.09.13 strain from the

252   initial isolate collection, showed 99% species identity with the unnamed Magnaporthaceae

253   sp. an uncultured *Phialophora* species isolated in 2009 (NCBI taxonomy ID: 268601) (Moll

254   et al. 2016), Magnaporthaceae sp. (NCBI taxonomy ID: 1940802) strains CPC 26284

255   (Hernández-Restrepo et al. 2016) and Magnaporthaceae sp. isolate 437 (Ulrich et al. 2000)

256   (Table 1 and Supplementary Table S4). Interestingly, both *G. hyphopodioides* and the

257   unnamed Magnaporthaceae sp. were isolated from the same field in the case of Summerdells

258   I, whereas only *G. hyphopodioides* was recovered from the other two fields (New Zealand

259   and Pastures).

260   Further isolates were obtained from colonised root tissue of the soil core bioassay plants from

261   the two experimental field trials in New Zealand field to confirm the presence of

262   *Gaeumannomyces* species. The ITS5-ITS4 rDNA sequences for all 19 isolates from the 2015

263   field trial and all 19 isolates from the 2016 field trial, also showed 99%-100% species identity

264 with the three *G. hyphopodioides* strains (CPC 26267, CPC 26249 and CPC 26248)

265 (Hernández-Restrepo et al. 2016), and were found to be the top three hits for all isolates from

266 the NCBI database (Table 1). The ITS5-ITS4 region was highly conserved across all *G.*

267 *hyphopodioides* isolates recovered in 2013, 2015 and 2016, with only one single nucleotide

268 polymorphism (SNP) across all 47 isolates.

269 A phylogenetic analysis was constructed to identify the genetic relationship between isolates

270 within the initial isolate collection (n= 9) and the isolates obtained from the two experimental

271 field trials (n= 38), as well as the relationship of these isolates to 32 reference isolates

272 downloaded from the NCBI database (Supplementary Table S4). The Magnaporthaceae sp.

273 isolate S.09.13, recovered from the initial 2013 isolate collection, clusters with all the

274 unnamed Magnaporthaceae sp. isolates in the NCBI database. The Magnaporthaceae sp. form

275 a separate clade from both *Gaeumannomyces* species (Fig. 2). The *G. hyphopodioides*

276 isolates recovered in 2013, 2015 or 2016 and reference NCBI isolates all cluster together,

277 separate from the *G. graminis* and *G. tritici* isolates obtained from NCBI (Fig. 2). Therefore,

278 this data confirms that the complete isolate collection contains two distinct species within the

279 Magnaporthaceae and these form two distinct clades, confirming the reassessed taxonomy of

280 the group by Hernández-Restrepo et al. (2016). Colonised seedling roots, from the initial

281 isolate collection, were examined under the light microscope and photographs were captured

282 to illustrate the two colonisation phenotypes identified (Fig. 1). The characteristic large,

283 single sub-epidermal vesicles were found for *G. hyphopodioides* colonised roots (Deacon

284 1974) (Fig. 1a) (isolate P.10.13 (Table 1)) and small clusters of sup-epidermal vesicles found

285 for colonised seedling roots by the unnamed Magnaporthaceae sp. (Ulrich et al. 2000) (Fig.

286 1b) (isolate S.09.13 (Table 1)).

287 *Cereal genotype root colonisation in seedling pot bioassay*

288 A seedling pot bioassay with artificial inoculum addition was devised to evaluate the ability

289 of the two fungal species within the Magnaporthaceae isolate collection to colonise the roots

290 of selected cereal species and wheat genotypes. Two experimental pot bioassays were carried

291 out and a significant interaction was identified between the percentage of colonised roots

292 between the two fungal species across the eight cereal genotypes (P<0.001) (Table 2). A

293 ~50% level of colonisation of the roots for the wheat cultivar Hereward was reached,

294 providing a benchmark to allow good discrimination. There was a statistically significant

295 difference in the main effect of percentage of roots colonised by the two fungal species in the

296 second pot bioassay (ANOVA: P <0.001, d.f. = 1, SED = 0.160) but not for the first pot
297 bioassay (ANOVA: P = 0.168, d.f. = 1, SED = 0.152). However, particularly noticeable was
298 the low level of fungal colonisation of oat roots for both species. A high level of fungal
299 colonisation was observed across the diploid wheat (*T. monococcum*), hexaploid wheat and
300 triticale cultivars, whereas in a take-all bioassay triticale is moderately resistant (McMillan et
301 al. 2011). For rye there was a low level of colonisation for the unnamed Magnaporthaceae
302 species but higher levels for *G. hyphopodioides*. Overall, the percentage of roots colonised by
303 the unnamed Magnaporthaceae sp. was statistically significantly higher than the percentage
304 of roots colonised by *G. hyphopodioides* for all cereal genotypes, except rye (Table 2) where
305 the reverse outcome was clearly evident. Representative colonisation phenotypes for both
306 species are shown in Fig. 1.

307 *Colonisation of UK winter wheat cultivars under field conditions*

308 The third aim of the study was to explore whether there were any differences in the ability of
309 current commercial UK winter wheat cultivars to support natural populations of *G.*
310 *hyphopodioides* in the field in a first wheat situation, measured by their colonisation on a
311 subsequent crop in the seedling soil core bioassay. Soil cores taken from the two field trials
312 and subsequently assessed in the seedling soil core bioassay, baited with Hereward, revealed
313 that there were differences between elite wheat cultivars (Fig. 4 and Supplementary Table S5
314 and Table S6). The overall level of *G. hyphopodioides* inoculum, measured by the percentage
315 of root colonisation of Hereward, differed across the two years. The field trial grand mean in
316 2016 (7.55%) was almost double the grand mean in 2015 (3.82%) (Supplementary Table S5).
317 Correlation between the two years was low (r_s = -0.04, P = 0.798) with many cultivars
318 showing contrasting results, for example, Hereward seedlings sown after Gallant had 5% of
319 roots colonised in the soil core bioassay in the 2015 field trial and 17% of colonised roots in
320 the 2016 field trial. However, there was a subset of cultivars which were consistently low in
321 supporting *G. hyphopodioides* inoculum in both years (for example Alchemy and Dickens),
322 as well as cultivars consistently supporting higher levels of inoculum in the two trial years
323 (Zulu, KWS Croft, KWS Kielder and KWS Sterling) (Supplementary Table S5). When data
324 was pooled from both years in a combined REML variance components analysis, there was
325 an overall significant effect of cultivar, revealing that Alchemy was the lowest supporter of
326 *G. hyphopodioides* inoculum, whereas KWS Kielder supported the highest levels of *G.*
327 *hyphopodioides* inoculum, 18% higher than for Alchemy (Fig. 3.). Eleven cultivars supported
328 higher levels of *G. hyphopodioides* inoculum than the control cultivar of Hereward (Fig. 3).

329   The fourth aim of this study was to establish whether there was any interaction between
330   second wheat cultivar choice, used as the baiting cultivar in the soil core bioassay, and their
331   subsequent level of root colonisation by *G. hyphopodioides*. To address this, half of the soil
332   cores were baited back on themselves with the same cultivar grown in the field trial and
333   compared to the cores previously baited with the highly take-all susceptible cultivar
334   Hereward. Most winter wheat cultivars were found to be poorly colonised by *G.*
335   *hyphopodioides* when baited with the same field plot cultivar (25/40 cultivars) in both
336   experiments (< 5 % roots infected (Fig. 4)). However, a subset of cultivars, including
337   cultivars Einstein, Solstice and JB Diego, KWS Kielder, Scout and Cordiale consistently had
338   higher levels (>10% of roots) of *G. hyphopodioides* root colonisation in both years (Fig. 4
339   and Supplementary Table S5). A strong correlation ($r_s = 0.765$, P<.001) between the two
340   years in the level of root colonisation by *G. hyphopodioides* was found, in contrast to the low
341   correlation found when baited with Hereward in aim three.

342   A significant interaction was found for the second wheat cultivar choice across the 40
343   cultivars (2015, *P* <0.001; 2016, *P* <0.001), with a trend for a higher percentage of roots
344   colonised with *G. hyphopodioides* when baited with Hereward for most elite winter wheat
345   cultivars (17 cultivars had 10% or more roots colonised with Hereward across one or both
346   field trials), with only eight cultivars giving a higher percentage of colonised roots when
347   baited with the field plot compared to when baited with Hereward (Supplementary Table S5).
348   The 25 winter wheat cultivars that were found to support low colonisation of *G.*
349   *hyphopodioides,* when the second wheat cultivar was the field plot cultivar, were found to
350   support higher levels of root colonisation when the second wheat cultivar was Hereward,
351   except for Alchemy (Supplementary Table S5). Inconsistencies in the level of root
352   colonisation between the two baiting methods is highly evident for cultivars Zulu, Leeds and
353   KWS Croft (Supplementary Table S5). In contrast, there were no cultivars that had a very
354   low percentage of root colonisation by *G. hyphopodioides* (<5%) when baited with Hereward
355   in the soil core bioassay, as well as having a moderate percentage of roots colonised when
356   baited with the field plot cultivar (Fig. 4). A pooled cross-season REML variance
357   components analysis across the 40 cultivars, revealed that nine cultivars supported medium
358   levels of *G. hyphopodioides* root colonisation (>10% roots colonised), regardless of second
359   wheat cultivar choice (Supplementary Table S6).

360   Although the field trial site has natural underlying populations of *G. hyphopodioides*, the soil
361   core bioassay plants were also assessed for any visible take-all infection. As expected there

362   was a negligible amount of take-all across the field site for both field trial years, with less

363   than 2.1% of roots infected with take-all across all cultivars (Supplementary Table S7 and

364   Supplementary Table S8).

365   The plot yields were taken from both experimental field trials and there were significant

366   effects of cultivar on plot yields for both field trial years (2015, $P <0.001$; 2016, $P <0.001$)

367   (Supplementary Table S9). No correlation was found between the plot yields and the

368   percentage of roots colonised with *G. hyphopodioides* when baited with Hereward, in the soil

369   core bioassay, for the 2015 field trial ($r_s= 0.102$, $P = 0.133$, n = 40), but a weak negative

370   correlation was found for the 2016 field trial ($r_s= -0.228$, $P = 0.039$, n = 40). No correlations

371   were found between the plot yields and the percentage of roots colonised with *G.*

372   *hyphopodioides* when baited with the field plot cultivar, in the soil core bioassay, for either

373   field trial year (2015: $r_s= -0.100$, $P = 0.134$, n = 40; 2016: $r_s= -0.099$, $P = 0.136$, n = 40).

374   **Discussion**

375   In this study, a new UK arable soil derived collection of *G. hyphopodioides* and

376   Magnaporthaceae sp. isolates was obtained over three cropping seasons and characterised

377   with existing information from the recent taxonomical reclassification of the

378   Magnaporthaceae family by Hernández-Restrepo et al. (2016). A seedling pot bioassay with

379   artificial inoculum addition then revealed that there were differences in the susceptibility of

380   five cereal species at the seedling stage to the two fungal species. The winter wheat cultivar

381   Hereward was found to be highly susceptible in the artificial pot bioassay, to both fungal

382   species, and was subsequently chosen to be used as the baiting cultivar in the seedling soil

383   core bioassay to test the difference between cultivars in their ability to support *G.*

384   *hyphopodioides* inoculum under field trial conditions. There was some evidence of a

385   difference between cultivars in their ability to support *G. hyphopodioides* inoculum under the

386   first wheat crop (gauged using Hereward as the baiting cultivar), although this was not very

387   consistent across the two trial years, indicating a strong genotype x environment component.

388   In contrast, there were more consistent differences between cultivars in the ability of *G.*

389   *hyphopodioides* to colonise seedlings in the soil core bioassay, when baited with the field plot

390   cultivar. We discovered that by changing the hexaploid wheat cultivar used as the bait in the

391   soil core bioassay, the level of *G. hyphopodioides* root colonisation was often altered.

392   Collectively, these new results provide valuable information on how beneficial soil dwelling

393 fungi can be encouraged to proliferate in arable soils to benefit wheat root health and hence
394 grain production.

395 The first aim of the study was to gather an isolate collection from arable fields on an
396 experimental farm in south-east England. There was a higher recovery of isolates of the *G.*
397 *hyphopodioides* species compared to the unnamed Magnaporthaceae sp. The two species
398 were only recovered together from one field, whereas in two other fields only *G.*
399 *hyphopodioides* was recovered. No isolates were recovered from the fourth sampled field
400 (Great Knott III) where beneficial Magnaporthaceae sp. had previously been visually
401 identified ~ 8 years previously (McMillan, personal communication). No isolates of
402 *Slopeiomyces cylindrosporus* (Klaubauf et al. 2014) (anamorph: *Phialophora graminicola*
403 (Walker 1980), previously isolated and studied in Rothamsted field trials (Ward and Gray
404 1992, Bryan et al. 1995) were isolated. Collectively these results indicate that the populations
405 of these soil dwelling beneficial fungal species are not static.

406 The 47 *G. hyphopodioides* isolates gathered from the various sites / trials across the
407 Rothamsted Farm, were found to be highly conserved across the ITS region. This isolate
408 collection is an important resource for future studies. Experiments are already underway to
409 sequence and fully assemble the genomes of different Magnaporthaceae species within the
410 collection and comparative studies with *G. tritici* should permit an improved understanding
411 of the key differences between these closely related soil dwelling beneficial and pathogenic
412 species. The isolate collection could also be used to design a species-specific diagnostic assay
413 to allow the identification of the different beneficial fungi present in arable fields.

414 The isolate collection was further used in the current study to establish a seedling pot
415 bioassay under controlled environmental conditions, with the aim of exploring the root
416 colonisation of different cereal species by non-pathogenic soil-borne Magnaporthaceae
417 species. Triticale had a high level of colonisation for both fungi, whereas triticale is
418 moderately resistant to the take-all fungus (McMillan et al. 2014). The remaining cereal
419 genotypes, including the ancestral wheat relative *T. monococcum* (A^m genome), the hexaploid
420 wheat landrace Watkins 1190777 and semi-modern elite spring and winter wheat genotypes
421 appeared to be equivalent in their level of fungal colonisation at the seedling stage. This
422 result suggests that fungal colonisation by beneficial *Gaeumannomyces* species has not been
423 significantly altered by intensive wheat breeding activities. Rye had a low level of root
424 colonisation by the unnamed Magnaporthaceae sp., suggesting that rye could be activating a

425 similar defence mechanism against the fungus as observed with take-all (Rothrock 1988).

426 The naïve soil used to establish all the pot bioassays was not sterilised, which explains why

427 very low levels of visible sub-epidermal vesicles could be found on the roots of the non-

428 inoculated Hereward control roots.

429 The third aim of this study was to investigate whether there were any differences in the

430 ability of current commercial UK winter wheat cultivars to support natural populations of *G.*

431 *hyphopodioides* in the field in a first wheat situation. The two years of Hereward baiting data

432 revealed that there were differences in the ability of the elite wheat cultivars to support *G.*

433 *hyphopodioides* inoculum under a first wheat crop. However, there were inconsistencies in

434 the level of root colonisation for cultivars between the two years, highlighting a genotype x

435 environment interaction. The higher level of *G. hyphopodioides* root colonisation in the 2016

436 field trial, suggests the 2015-2016 season was more environmentally conducive to supporting

437 natural populations of *G. hyphopodioides*. Weather conditions in 2015 consisted of a wet

438 spring and summer compared to a drier spring and summer in 2016 (Supplementary Table

439 S10). This contrasts with take-all disease which is generally favoured by warmer winters and

440 wet springs/summers. Alternatively, differences in field site location may account for

441 differences in levels of *G. hyphopodioides* inoculum between the two years.

442 The wheat genotype-*G. hyphopodioides* interaction detected in aim three of this study

443 complements an earlier study that had identified consistent differences in the ability of wheat

444 cultivars to build-up take-all (*G. tritici*) inoculum under a first wheat crop, named the take-all

445 inoculum build-up trait (TAB) (McMillan et al. 2011). However, there was no clear

446 correspondence between the previously described TAB phenotypes of Cadenza and

447 Hereward, low and high TAB respectively, and their ability to support populations of *G.*

448 *hyphopodioides* in this study (11.9% and 14.7% roots colonised with *G. hyphopodioides*

449 when baited with Hereward in the soil core bioassay, Supplementary Table S6)

450 Finally, the fourth aim was to establish whether there was any interaction between second

451 wheat cultivar choice and level of root colonisation by *G. hyphopodioides*. The majority of

452 cultivars were found to support low levels of root colonisation, when the field plot cultivar

453 represented the subsequent second wheat, rather than Hereward. But significant interactions

454 were also evident. Nine cultivars across the two years consistently exhibited the ability to

455 support medium levels of *G. hyphopodioides* root colonisation, independent of second wheat

456 choice. For example, the elite cultivars Scout and KWS Kielder, indicated the highest level of

457   *G. hyphopodioides* root colonisation, regardless of the second wheat cultivar choice.

458   Whereas, the cultivar Alchemy, consistently had the lowest level of *G. hyphopodioides* root

459   colonisation across the two second wheat cultivar choice. Whilst cultivars Zulu, Leeds and

460   KWS Croft indicated contrasting results from the two baiting methods.

461   Collectively, these data provide the first evidence for complex host genotype-*G.*

462   *hyphopodioides* interactions occurring under both arable field conditions and in the five week

463   seedling pot bioassay. The seedling pot bioassay screened a wide variety of cereal germplasm

464   and cultivar, both modern and historical, yet there was little difference in the ability of *G.*

465   *hyphopodioides* to colonise the roots of this diverse wheat germplasm under artificial

466   conditions. The soil core bioassay from the experimental field trials, screened less diverse

467   modern wheat cultivars and revealed statistically significant differences in the ability of these

468   cultivars to be colonised and also to support natural populations of *G. hyphopodioides* in the

469   soil. These data suggest that wheat plants at the seedling stage may differ in their interaction

470   with *G. hyphopodioides* during root colonisation compared to adult plants in the field. The

471   significantly different results obtained using the two baiting methods supports the suggestion

472   that fungal inoculum presence (measured using Hereward as the baiting cultivar) is an

473   independent trait to seedling root colonisation. It is highly likely that the two phenomena are

474   controlled by different mechanisms and may involve interactions with other soil dwelling

475   microbes and / or root exudates.

476   High extrapolated yield data was calculated for both field experiments (2015 range: 17.83-

477   25.57 tonnes/hectare; 2016 range: 12.68-22.93 tonnes/hectare, Supplementary Table S9).

478   There appears to be no strong evidence of a detrimental effect of *G. hyphopodioides*

479   colonisation on the yield of the plots. This complements field trials conducted in Australia

480   investigating the cross-protection of *G. hyphopodioides* against take-all disease (Wong et al.

481   1996).

482   One *G. hyphopodioides* isolate has been patented for take-all control in Australia (Wong et al.

483   1996). No commercial use has been documented and pelleting wheat seeds with *G.*

484   *hyphopodioides* is not currently utilised as a method of biological control against take-all

485   disease. The percentage of UK fields that contain this beneficial organism is unknown.

486   However, this soil-borne species has been documented worldwide, including the USA,

487   Australia, Poland and Germany and was identified in three of the four suppressive field sites

488   on the arable farm used for this study. The ability of elite winter wheat cultivars to support

489 and be colonised by natural populations of *G. hyphopodioides* under a first wheat crop

490 suggests important host genotype-fungal interactions which, if harnessed, could potentially

491 provide an additional management strategy, not only in the UK, to help combat take-all root

492 disease in second wheats.

493 From a wheat breeding perspective, there does not appear to be any interaction between *G.*

494 *hyphopodioides* root colonisation and the National Association of British and Irish Flour

495 Millers (nabim) groupings or pedigrees of the elite wheat cultivars. For example, the Robigus

496 pedigree is found in several cultivars within the AHDB 2013/2014 RL and lines from the

497 2014/2015 RL winter wheat cultivars, yet there appears to be no similarities across these

498 cultivars in their level of *G. hyphopodioides* root colonisation with either of the second wheat

499 cultivar choices. This suggests the trait is not under simple genetic control and could also be

500 influenced by environmental factors such as soil type, soil moisture and biological factors

501 such as the overall make-up of the rhizosphere/soil microbiome. However, consistent

502 differences across the two field seasons were observed for a subset of nine cultivars,

503 suggesting that suitable mapping populations could be generated to investigate the genetic

504 basis of these interactions.

505 In summary, this is the first report of two robust field trial datasets that have revealed UK

506 elite winter wheat cultivars differ in their ability to support and be colonised by natural

507 populations of the take-all root disease supressing fungus, *G. hyphopodioides*, under a first

508 wheat crop. Although there were some clear inconsistencies between field seasons, this dual

509 dataset reveals that a sub-set of nine elite UK winter wheat cultivars consistently supported

510 fungal inoculum and seedling root colonisation by *G. hyphopodioides*. These cultivars have

511 the potential to be used to encourage populations of introduced or resident beneficial fungi

512 for the control of take-all disease in short wheat rotations. Further research is now required to

513 explore the genetic and mechanistic basis of this interaction and the influence of

514 environmental and genetic factors on soil population establishment, root colonisation and

515 take-all control.

516 **Supplementary Data**

517 **Supplementary Fig. S1.** Previous field trial sites on the Rothamsted Farm that have shown
518 suppression of take-all disease in experiments

519 **Supplementary Table S1.** Soil core sampling details for establishing the isolate collection

520   **Supplementary Table S2.** Experimental field trial details to evaluate the ability of elite UK

521   winter wheat cultivars to support *Gaeumannomyces hyphopodioides* inoculum under a first

522   wheat crop across the two field seasons.

523   **Supplementary Table S3.** Details of fertiliser, pesticide and growth regulator applications

524   to the two experimental field trials.

525   **Supplementary Table S4**. GenBank accession numbers for sequences used in the

526   phylogenetic analysis.

527   **Supplementary Table S5.** Percentage of roots colonised with *Gaeumannomyces*

528   *hyphopodioides* in the soil core bioassay for the two field trials in 2015 and 2016.

529
530   **Supplementary Table S6.** Combined REML variance components analysis of mean

531   percentage of roots colonised with *Gaeumannomyces hyphopodioides* in the soil core

532   bioassay for the two field trials in 2015 and 2016.

533   **Supplementary Table S7.** Percentage of roots infected with take-all (*Gaeumannomyces*

534   *tritici*) in the soil core bioassay for the two field trials in 2015 and 2016.

535   **Supplementary Table S8.** Combined REML variance components analysis of mean

536   percentage of roots colonised with take-all (*Gaeumannomyces tritici*) in the soil core bioassay

537   for the two field trials 2015 and 2016.

538   **Supplementary Table S9.** Grain yield for elite wheat cultivars for each experimental field

539   trial and mean grain yields across two field trials analysed by a combined REML variance

540   components analysis.

541   **Supplementary Table S10.** Monthly rainfall (mm) and maximum daily temperature (°C) for

542   the months of May-August during the two field trial seasons of 2015 and 2016 (data

543   downloaded from the electronic Rothamsted Archive (e-RA)).

558

559   **Author contributions**

560   SJO, VM and KHK designed the research. SJO and VM conducted the research. SJO and RW

561   analysed the data. SJO, VM and KHK wrote the manuscript.

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Table 1. Fungal isolate identity in the initial collection from the field season year 2013 and isolates obtained from the two experimental field trials in the field season years 2015 and 2016.

Isolate code	Original field host and cultivar	Soil bioassay host and cultivar	RRes field name	Fungal identity
Initial isolate collection[1]				
N.14.13[2,3]	*Hordeum vulgare,* Tipple	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
N.20.13	*Hordeum vulgare,* Tipple	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
P.03.13	*T. aestivum,* Conqueror	*T. aestivum*, Hereward	Pastures	*G. hyphopodioides*
P.05.13	*T. aestivum,* Conqueror	*T. aestivum*, Hereward	Pastures	*G. hyphopodioides*
P.06.13	*T. aestivum,* Conqueror	*T. aestivum*, Hereward	Pastures	*G. hyphopodioides*
P.09.13	*T. aestivum,* Conqueror	*T. aestivum*, Hereward	Pastures	*G. hyphopodioides*
P.10.13	*T. aestivum,* Conqueror	*T. aestivum*, Hereward	Pastures	*G. hyphopodioides*
S.03.13	*T. aestivum,* Conqueror	*T. aestivum*, Hereward	Summerdells I	*G. hyphopodioides*
S.09.13[4]	*T. aestivum,* Conqueror	*T. aestivum*, Hereward	Summerdells I	Magnaporthaceae sp.
2015/R/WW/1516 field trial				
NZ.16.1A[5].15	*T. aestivum*, Zulu	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.24.2A.15	*T. aestivum*, KWS Kielder	*T. aestivum*, KWS Kielder	New Zealand	*G. hyphopodioides*
NZ.112.1A.15	*T. aestivum*, KWS Target	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.136.1A.15	*T. aestivum*, Tuxedo	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.141.2A.15	*T. aestivum*, Duxford	*T. aestivum*, Duxford	New Zealand	*G. hyphopodioides*
NZ.155.1A.15	*T. aestivum*, Revelation	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.160.1A.15	*T. aestivum*, KWS Sterling	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.173.2A.15	*T. aestivum*, Solstice	*T. aestivum*, Solstice	New Zealand	*G. hyphopodioides*
NZ.8.1B.16	*T. aestivum*, Delphi	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.12.2B.16	*T. aestivum*, Solstice	*T. aestivum*, Solstice	New Zealand	*G. hyphopodioides*
NZ.110.2B.16	*T. aestivum*, Cordiale	*T. aestivum*, Cordiale	New Zealand	*G. hyphopodioides*
NZ.43.1C.16	*T. aestivum*, Relay	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.93.2C.16	*T. aestivum*, JB Diego	*T. aestivum*, JB Diego	New Zealand	*G. hyphopodioides*
NZ.103.2C.16	*T. aestivum*, Solstice	*T. aestivum*, Solstice	New Zealand	*G. hyphopodioides*
NZ.136.1C.2.16	*T. aestivum*, Tuxedo	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.138.1C.16	*T. aestivum*, Zulu	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.176.1C.16	*T. aestivum*, Evolution	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.183.1C.16	*T. aestivum*, Invicta	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.184.1C.16	*T. aestivum*, Monterey	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*

Table 1. Continued.

Isolate code	Original field host and cultivar	Soil bioassay host and cultivar	RRes field name	Fungal identity
2016/R/WW/1620 field trial				
NZ.3.2A.17	*T. aestivum*, Scout	*T. aestivum*, Scout	New Zealand	*G. hyphopodioides*
NZ.143.1A.17	*T. aestivum*, KWS Croft	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.198.1A.17	*T. aestivum*, Invicta	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.38.1B.17	*T. aestivum*, KWS Sterling	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.46.1B.17	*T. aestivum*, Relay	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.86.2B.17	*T. aestivum*, Einstein	*T. aestivum*, Einstein	New Zealand	*G. hyphopodioides*
NZ.109.1B.17	*T. aestivum*, Grafton	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.114.2B.17	*T. aestivum*, KWS Gator	*T. aestivum*, KWS Gator	New Zealand	*G. hyphopodioides*
NZ.148.1B.17	*T. aestivum*, Relay	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.164.1B.17	*T. aestivum*, Monterey	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.185.2B.17	*T. aestivum*, Cordiale	*T. aestivum*, Cordiale	New Zealand	*G. hyphopodioides*
NZ.23.1C.17	*T. aestivum*, Viscount	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.41.2C.17	*T. aestivum*, KWS Gator	*T. aestivum*, KWS Gator	New Zealand	*G. hyphopodioides*
NZ.104.1C.17	*T. aestivum*, KWS Sterling	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.115.1C.17	*T. aestivum*, KWS Target	*T. aestivum*, Hereward	New Zealand	*G. hyphopodioides*
NZ.129.2C.17	*T. aestivum*, Scout	*T. aestivum*, Scout	New Zealand	*G. hyphopodioides*
NZ.135.2C.17	*T. aestivum*, Solstice	*T. aestivum*, Solstice	New Zealand	*G. hyphopodioides*
NZ.155.2C.17	*T. aestivum*, Cordiale	*T. aestivum*, Cordiale	New Zealand	*G. hyphopodioides*
NZ.160.2C.17	*T. aestivum*, Cadenza	*T. aestivum*, Cadenza	New Zealand	*G. hyphopodioides*

[1] No isolates were recovered from Great Knott III RRes field on the Rothamsted Farm.

[2] Year of isolation is represented by the last two digits of the isolate ID, e.g. N.14.13 was isolated in 2013.

[3] *Gaeumannomyces hyphopodioides* isolate N.14.13 was used in the pot bioassay to screen the susceptibility of different cereal species and genotypes.

[4] Magnaporthaceae sp. isolate S.09.13 was used in the pot bioassay to screen the susceptibility of different cereal species and genotypes.

[5] The post-harvest soil core bioassays from the two field trials were split into three groups to give one pot replicate per plot per group, when assessing the roots for *G. hyphopodioides* colonisation and therefore the codes A, B, C represent isolates from each of the three groups.

Table 2. Ability of *Gaeumannomyces hyphopodioides* and Magnaporthaceae sp. to colonise cereal roots in a potato dextrose agar (PDA) inoculated seedling pot bioassay in soil.

| Fungal species | Cereal genotype and cultivar | Logit percentage of colonised roots (back-transformed means) | | | |
		First pot bioassay		Second pot bioassay	
Gaeumannomyces hyphopodioides	Oats, Gerald	-4.05	(1.23)	-3.68	(1.99)
	Rye, Carotop	-0.88	(29.17)	-1.11	(24.48)
	Triticale, Trilogie	-0.26	(43.56)	-1.42	(19.22)
	T. aestivum, Hereward	-0.41	(39.82)	-0.37	(40.79)
	T. aestivum, Hereward -[1]	-2.12	(10.29)	-5.30	(0)
	T. aestivum, Paragon	-0.83	(30.14)	-1.03	(26.68)
	T. aestivum, Watkins 1190777	-0.37	(40.75)	-0.10	(48.13)
	T. monococcum, MDR037	-0.68	(33.42)	-0.07	(33.30)
	T. monococcum, MDR046	-0.23	(44.15)	-0.69	(26.05)
Unnamed *Magnaporthaceae species*	Oats, Gerald	-2.72	(5.75)	-2.40	(7.89)
	Rye, Carotop	-2.99	(4.33)	-2.63	(6.27)
	Triticale, Trilogie	-0.01	(49.76)	-0.06	(48.61)
	T. aestivum, Hereward	0.03	(50.69)	0.41	(60.11)
	T. aestivum, Hereward -[1]	-1.85	(13.22)	-4.37	(0.76)
	T. aestivum, Paragon	-0.65	(34.16)	0.24	(57.35)
	T. aestivum, Watkins 1190777	0.09	(52.34)	0.29	(66.20)
	T. monococcum, MDR037	0.33	(58.32)	0.67	(53.28)
	T. monococcum, MDR046	-0.15	(46.35)	0.13	(56.13)
	d.f.	8		8	
	SED (logit scale)	0.455		0.481	
	F Probability	<0.001		0.005	

[1] Hereward - = Hereward negative control with non-colonised PDA. Microscopic analysis revealed very small clustered sub-epidermal vesicles and the species is thought to either the unnamed Magnaporthaceae sp. or *Slopeiomyces cylindrosporus* (NCBI Taxonomy ID: 1577607) (Klaubauf et al. 2014), unfortunately this isolate was not recovered.

Fig. 1. *Gaeumannomyces hyphopodioides* colonised wheat (cultivar Hereward, isolate P.10.13) seedling root (a). White arrow indicates the colonisation phenotype of large, single sub-epidermal vesicles, magnification X67. Unnamed Magnaporthaceae sp. colonised wheat (cultivar Hereward, isolate S.09.13) seedling root (b), magnification X92.3. White arrows indicate the colonisation phenotype of small and clustered sub-epidermal vesicles.

Fig. 2. Phylogenetic tree of the ITS5-ITS4 rDNA regions of isolates from the initial isolate collection and *Gaeumannomyces hyphopodioides* isolates from the two experimental field trials, along with sequences obtained from the NCBI database of species within Magnaporthaceae. The genetic distance model Tamura-Nei was used and a tree build method of Neighbor-joining performed with 100 bootstraps. A 75% support threshold was used.

Fig. 3. Percentage of roots colonised with *Gaeumannomyces hyphopodioides* (back-transformed means of the logits) when baited with the winter wheat cultivar Hereward in the soil core bioassay. Combined analysis of data pooled across the two years (Chi squared probability <0.001, SED (logit scale)= 0.171, Wald statistic= 637.76). See Supplementary Table S5 for data on logit scale.

Fig. 4. Correlation between percentage of roots colonised with *Gaeumannomyces hyphopodioides* (back-transformed means of the logits) when baited with the field plot cultivar or Hereward in the soil core bioassay in 2015 (a) (P <0.001, SED (logit scale)= 0.231, F statistic= 5.58) and 2016 (b) (P <0.001, SED (logit scale)= 0.194, F statistic= 13.50). Key: Be= Beluga, Cc= Cocoon, Cf= KWS Croft, Cg= Cougar, Ch= Chilton, Cl= Claire, Cn= Conqueror, Cr= Crusoe, De= Delphi, Di= Dickens, Dn= Denman, Ev= Evolution, Ho=Horatio, In= Invicta, Le=Leeds, Mo= Monterey, My= Myriad, Re= Revelation, Sa= KWS Santiago, St= KWS Sterling, Ta= KWS Target, Tu= Tuxedo and Vi= Viscount. Very low root colonisation: <5%, low root colonisation: 5-10%, medium root colonisation: >10%.