Yield losses and control by sedaxane and fludioxonil of soil-borne *Rhizoctonia*, *Microdochium*, and *Fusarium* species in winter wheat

Matthew Brown[1], Dasuni P. Jayaweera[1], Annabel Hunt[1], James W. Woodhall[2], and Rumiana V. Ray[1*]

[1]Division of Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire, LE12 5RD, UK

[2] University of Idaho, Parma Research and Extension Center, Idaho, USA

*Corresponding author. E-mail: rumiana.ray@nottingham.ac.uk

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Abstract

Soil-borne *Rhizoctonia*, *Microdochium*, and *Fusarium* species are major causal agents of seedling and stem-base diseases in wheat, and currently seed treatments are considered the most effective solution for their control. *Rhizoctonia solani* anastomosis groups (AGs) 2-1 and 5, *R. cerealis*, *Microdochium*, and *Fusarium* spp. were used in series of field experiments to determine their capability to cause soil-borne and stem-base disease and to quantify their comparative losses in establishment and yield of wheat. The effectiveness and the response to seed treatment formulated of 10 g sedaxane and 5 g fludioxonil 100 kg^{-1} against these soil-borne pathogens were also determined. Our results showed that damping off caused by soil-borne *R. cerealis* was associated with significant reductions in emergence and establishment resulting in stunted growth and low plant numbers. The pathogen also caused sharp eyespot associated with reductions in ear partitioning index. *R. solani* AG 2-1 or AG 5 were weakly pathogenic and failed to cause significant damping off, root rot, or stem-base disease in wheat. *Fusarium graminearum* and *F. culmorum* applied as soil-borne inoculum failed to cause severe disease. *Microdochium* spp. caused brown foot rot disease and soil-borne *M. nivale* reduced wheat emergence. Application of sedaxane and fludioxonil increased plant emergence and reduced damping off, early stem-base disease, and brown foot rot, thus providing protection against multiple soil-borne pathogens. *R. cerealis* reduced thousand grain weight by 3.6% whilst seed treatment of fludioxonil and sedaxane against soil-borne *R. cerealis* or *M. nivale* resulted in 4% yield increase.

Keywords: soil-borne disease, *Rhizoctonia* spp., *Microdochium* spp., *Fusarium* spp., seed treatment, wheat yield

Introduction

Wheat (*Triticum aestivum*) is the most widely grown crop in the UK on approximately 1.7 million ha with total production of 18 Mt annually (DEFRA 2019). Intense wheat rotations lead to build up of soil-borne diseases associated with reductions of yield due to loss in early plant establishment and disease-imposed limitations on root and stem water/nutrient uptake (Oerke 2006). The soil-borne pathogens that occur most commonly in short wheat rotations in the UK include *Rhizoctonia solani* Anastomosis group (AG) 2-1, AG 5, *Rhizoctonia cerealis* BNR AG-D (Brown et al. 2020), *Microdochium*, and *Fusarium* species (Turner et al. 2002).

In UK wheat crops, *R. cerealis* is predominantly associated with the stem-base disease sharp eyespot (Hardwick et al. 2001; Parry 1990) resulting in pre-mature ripening, shrivelled grains, and lodging (Lemańczyk and Kwaśna 2013). The most recent yield losses due to sharp eyespot were estimated at 18% and 8-10% in New Zealand (Cromey et al. 2002) and in Poland (Lemańczyk and Kwaśna 2013), respectively. There is generally low awareness by growers/agronomists in the UK of the capability of *R. cerealis* to cause pre- and post-emergence damping-off (Parry 1990), and the effects of this pathogen on emergence and establishment losses have not been previously investigated. Similar to *R. cerealis*, *Fusarium* and *Microdochium* spp. are adapted to wheat, causing three diseases within the Fusarium complex in cereals. Seed or soil-borne infections develop into Fusarium seedling blight (FSB) which can transition into brown foot rot (BFR) and then to Fusarium head blight (Glynn et al. 2007). FSB arising from seed infection is known to reduce seed germination capacity leading to poor crop establishment of wheat (Haigh et al. 2009). However, yield loss due to FSB from soil-borne infection remains unknown. AG 2-1 and AG 5 of *R. solani* have a diverse host range and cause predominantly pre- and post-emergence damping off on seedlings (Hamada et al. 2011a). Published pathogenicity experiments using wheat seedlings

grown under controlled environment (Demirci 1998; Roberts and Sivasithamparam 1986; Rush et al., 1994; Sturrock et al., 2015) demonstrate significant variation in virulence of their isolates, but evidence of their ability to cause significant disease in field-grown wheat that may result in yield loss is lacking. Some of these soil-borne pathogens occur in complexes that are confounded to specific tissues of the wheat host, for example BFR and sharp eyespot are part of the stem-base disease complex in cereals. Current knowledge of the symptoms and field yield losses caused by soil-borne pathogens is essential for growers and agronomists to optimize disease control as part of crop management.

Cultural control methods are not consistently effective in intensive wheat cropping, and thus seed treatments are the most reliable method for protecting seed germination and plant seedling growth when plants are most susceptible to soil-borne pathogens (Haigh et al. 2009; Zeun et al. 2013). The broad-spectrum fungicide sedaxane developed by Syngenta Crop Protection inhibits the succinate dehydrogenase enzyme in complex II of the mitochondrial respiration chain (Zeun et al. 2013). Glasshouse studies have shown that sedaxane has activity against *R. solani* on several crop species and field plots artificially inoculated with *R. cerealis* showed a yield advantage from treatments containing sedaxane (Zeun et al. 2013). Fludioxonil, applied as a seed treatment, has been shown previously to significantly reduce *Microdochium* and *Fusarium* DNA in seedlings achieving > 90% control against *F. culmorum, M. nivale*, *and M. majus* in field (Glynn et al. 2007). *R. cerealis* has also proved sensitive to fludioxonil *in vitro* (Hamada et al. 2011b). Based on the activity profiles of sedaxane and fludioxonil, a seed treatment containing the active fungicides will be potentially effective against the main soil-borne pathogen complex that may pose early threat to wheat in the UK.

In this study therefore, we aimed to determine the yield losses and effectiveness of seed treatments containing sedaxane and fludioxonil against the main soil-borne pathogens found in English wheat fields. The main objectives were to i) quantify the disease effects of soil-borne *R. solani* AG5, AG2-1, and *R. cerealis* on the host from emergence through to harvest yield, ii) compare yield loss due to the most pathogenic *Rhizoctonia* spp. with soil-borne *Fusarium graminearum*, *F. culmorum*, or *Microdochium nivale*, and iii) determine the effectiveness of fungicide seed treatments on disease severity and yield response.

Materials and methods

Experimental design. Two series of field experiments repeated over two years were carried out. The first series of field experiments were performed in 2012/13 and in 2013/14 to determine the effects of different *Rhizoctonia* spp. on early emergence, establishment, and yield. The second series of experiments in 2016/17 and in 2017/18 focussed on comparative yield losses between *R. cerealis*, identified as the most aggressive *Rhizoctonia* spp. from the first field experiments, and *Fusarium* and *Microdochium* spp. The effectiveness of fludioxonil used alone was tested only in the first series of experiments focussed on *Rhizoctonia* spp. as this fungicide is already known to be effective in field as a seed treatment against *Microdochium* and *Fusarium* spp. in wheat. Furthermore, since sedaxane is commercially available in a formulation with fludioxonil, only the formulated seed treatment was included in the second series of experiments.

The first field series of experiments with winter wheat cv. Santiago were designed as randomised block with two factors, pathogen inoculation (not-inoculated control, AG 2-1, AG 5, or *R. cerealis*) and seed treatment (untreated, fludioxonil (5 g a.i 100 kg^{-1}), or sedaxane (10 g a.i

100 kg^{-1}) + fludioxonil (5 g a.i 100 kg^{-1})) allowing twelve treatment combinations with four and three replications in 2012/13 and in 2013/14, respectively. The second series of field experiments with winter wheat cv. Leeds were also designed as randomised block with two factors, pathogen inoculation (*R. cerealis*, *F. graminearum* and *F. culmorium*, or *M. nivale*) and seed treatment (untreated or sedaxane (10 g a.i 100 kg^{-1}) + fludioxonil (5 g a.i 100 kg^{-1}) allowing six treatment combinations with three replications in 2016/17 and in 2017/18.

Agronomy. All field experiments were conducted at the University farm, Sutton Bonington, UK where winter wheat was sown in October at a standard rate of 320 seeds m^2. Inoculum grown on millet seed was drilled with the wheat seed at a rate of 30 g m^{-2} using a Wintersteiger plot drill. Plot size was 6 x 1.6 m in 2012/13, 1 x 1 m in 2013/14 and 12 x 1.6 m in 2016/17 and 2017/18. Crop protection followed standard agronomic practices except for the fungicide programme which was designed to give robust protection against foliar diseases and true eyespot utilising active substances that were not active towards *R. solani*, *R. cerealis*, *F. graminiarum*, *F. culmorum*, and *M. nivale* (Tables S1 and S2).

Inoculum preparation. Inoculum was grown on millet seed following the method described by Zeun et al. (2013). Isolates *R. solani* AG 2-1 (isolate 1917), AG 5 (isolate 1906), *R. cerealis* (Rc isolate 1480), *F. graminiarum* (isolates 13, 15 and 16), *F. culmorium* (isolates 218 and 236), and *M. nivale* (isolates 251, 252 and 253) taken from the University of Nottingham isolate collection were raised onto potato dextrose agar plates for inoculum production.

Mixed inoculum for plot application of *Fusarium* and *Microdochium* spp. where more than one isolate was included was prepared by adding individual isolate-inoculated millet seed in equal ratios. All isolates were of known pathogenicity to wheat. Inoculum was dry, and visually

inspected for adequate fungal colonization and lack of contamination prior to drilling with the wheat seed.

Plant sampling and crop assessments in the first series of experiments. Crop growth stages were assessed according Zadoks et al. (1974). Individual crop growth and physiological measurements, and disease assessments were performed at growth stage (GS) 15, GS 31, GS 75, and GS 85 for the first series of experiments. At GS 15 and GS 31, fifteen plants were randomly collected per plot for assessments. Later at GS 39 and GS 75, all plants were removed from within an area of 0.25 m^2 quadrat, placed at random per plot, and subjected to detailed biomass assessment. 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.

Seedling emergence and assessment of plant populations. Seedling emergence counts were taken periodically after sowing in the first series of experiments and plant numbers recorded at 29 days post soil inoculation (dpi) in the second series of experiments. Emerged seedlings were counted within a 0.25 m^2 quadrat (0.5 x 0.5 m) in three replicates per plot. This was then converted into a percentage of the 320 seeds m^2 sown. Plant numbers at GS 39 were counted per m^2 per plot in the first experiment.

Visual disease assessments. In the first series of experiments, 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 following classification of root rot disease assessment key described by Strausbaugh et al. (2004) and classification of eyespot, sharp eyespot and BFR severity described by Scott and Hollins (1974). In the second series of experiments, assessments were made at GS 15. 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. At GS 15, it was not possible to distinguish between stem-base diseases, therefore stems were assessed for generally stem browning based Scott and Hollins (1974) disease assessment key.

Pathogen DNA extraction and quantification using real-time PCR. Pathogen DNA extraction and quantification using real-time PCR were performed for the first series of experiments. DNA extraction from soil was only conducted on samples (50 g) collected at GS 15. Soil DNA was extracted throughout the period of this study using the method developed by Woodhall et al. (2012). Stem samples collected at GS 15 were extracted for DNA using BIOREBA® extraction bags due to the small sample size. Stems (5 cm stem basal region) from 15 plants were weighed and then placed in a BIOREBA® extraction bag. Samples were frozen in liquid nitrogen and cetyltrimethylammonium bromide (CTAB) buffer (6 ml) was added to the bag before macerating using a BIOREBA Homex 6 flatbed grinder. The resulting supernatant was centrifuged at 2000 x g for 2 minutes, and 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 13000 x g for 5 minutes. The resulting supernatant (500 µl) was used for extraction according to Wizard food kit (Promega) manufacturer's instructions in combination with 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 was extracted as described by Ray et al. (2004).

Real-time PCR assays were performed for AG 2-1, AG 5, and *R. cerealis* from DNA extracted from plants grown in plots inoculated with the aforementioned pathogens. Primers and probes used in this study are shown in Table S3. The qPCR conditions used are as described by Woodhall et al. (2017) for *Rhizoctonia* spp. and Nielson et al. (2013) for *Microdochium* spp.

Plant height, green area index, and ear partitioning index. Plant height (mm) from the stem base to the top of the longest leaf and green area index at GS 15, dry weight and ear partitioning index were performed in the first series of experiments. At 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, 2nd 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 later. 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 is a precise way of estimating the light-capturing capacity of a canopy (Pask et al. 2012). The green area index (GAI) was calculated using the following equation:

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

Ear partitioning index (EPI) defined as the fraction of above-ground DW at GS75 in the ear used the following equation:

$$EPI\ (GS\ 75) = DWE/\ (DWL+DWS+DWE) \quad (2)$$

Yield components. 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 (t ha^{-1}). Grain samples were used to quantify thousand grain weight (TGW) using a Sinar (model - AP6060-001AG) moisture analyser.

Statistical analysis. All data were analyzed using analysis of variance (ANOVA) with Genstat® Version 17.1 for Windows (VSN International Ltd, UK). DNA data was log_{10} transformed and disease index, where required, was angular transformed to normalize residuals. Back-transformed means of transformed data are presented in parentheses. Relationships between disease index and pathogen DNA were analyzed using regression analysis. Season (year of experimentation) was included in the treatment structure of ANOVA and results are presented for significant interactions or in their absence for the main effects of factors in the analysis.

Results

Effect of *Rhizoctonia* spp. and seed treatment on emergence and plant populations. There were no interactions between factors, including season, for emergence and plant numbers, thus the main significant effects are presented in Fig. 1. A significant and consistent decrease of plant emergence was observed under inoculation with *R. solani* AG 2-1 and *R. cerealis* compared to *R.*

solani AG 5 at 18 dpi ($P = 0.019$) (Fig. 1a). Reductions of 14% were observed in inoculated plots of *R. cerealis* compared to the not-inoculated control by 26 dpi. Fludioxonil alone or applied with sedaxane increased emergence (Fig. 1b) and plant numbers at GS39 (Fig. 1c) by 33% and 15%, respectively.

Rhizoctonia diseases and effects of seed treatments. Fludioxonil and fludioxonil + sedaxane reduced root rot symptoms by 29.1% and 35.1%, respectively, compared to the untreated (Fig. 2a). At GS 31, there was an interaction between treatment and season ($P = 0.046$) indicating that the control of root rot was not consistent in the two seasons of experimentation and in 2013/14, and root rot disease was significantly higher in fludioxonil (20%) treated plots than in the untreated (13.6%) (Fig. 2b). At GS 75, there was also an interaction between inoculation and treatment ($P = 0.038$) with higher root rot disease in AG 5 inoculated plots following treatment with fludioxonil compared to untreated (Fig. 2c). Fludioxonil reduced root rot disease index in the not-inoculated control and in *R. cerealis*- inoculated plots, whilst the addition of sedaxane contributed to a decrease in root rot under AG 2-1 inoculation compared to the untreated (Fig. 2c).

Stem-base diseases and effects of seed treatments. Due to the difficulty in identifying the early symptoms of individual stem-base diseases at GS 15, stems were assessed for general stem browning (Table 1). The interaction between inoculation and season was significant ($P = 0.002$) showing that AG 5 caused greater stem browning compared to the not-inoculated control in 2012/13. In both seasons, the highest stem browning at GS 15 occurred in *R. cerealis* inoculated plots. There was also stem browning in the not-inoculated control in 2012/13 suggesting that other pathogens were present. Real-time PCR revealed that these symptoms were associated with *Microdochium* spp. Seed treatments reduced stem browning ($P < 0.001$), and the interaction with season was significant ($P < 0.001$) due to fludioxonil not controlling stem browning in 2013/14.

In contrast, sedaxane + fludioxonil significantly reduced stem browning compared to the untreated by 47% and 38% in 2012/13 and 2013/14, respectively (Table 1).

Sharp eyespot and BFR were assessed at GS 31, 39, and 75 (Fig. 3). Sharp eyespot index was highest in the *R. cerealis*-inoculated plots throughout the assessment period ($P < 0.001$) (Fig. 3a). Slight symptoms were observed in AG 2-1 (2.1-5.1%) and AG 5 (3-5%) inoculated plots and the not-inoculated control plots (3-5.7%). There was no effect of seed treatments on sharp eyespot disease (data not presented).

Brown foot rot index was significantly ($P < 0.001$) lower in *R. cerealis* (8.4-15.6%) inoculated plots than AG 2-1 (20.4-27.5%), AG 5 (21.1-27.5%), and not-inoculated control plots (25.8-28.5%) (Fig. 3b). Sedaxane + fludioxonil reduced BFR by 30.4% and 23% compared to the untreated at GS 31 and GS 39, respectively (Fig. 3c). There was no seed treatment effect present at GS 75.

Effect of inoculation and seed treatment on *Rhizoctonia* spp. DNA in soil and *in planta*. Pathogen DNA in soil samples was quantified at GS 15 (Table 2). The highest DNA concentrations were quantified in the inoculated untreated plots. DNA of *R. cerealis* was found at > 4000 pg g^{-1} of soil, followed by AG 2-1 DNA > 220 pg ng^{-1} of soil and then by DNA of AG 5, which differed significantly in 2012/13 and 2013/14 at 141 and 0.10 pg g^{-1} of soil, respectively (Table 2). DNA of AG 2-1, AG 5, and *R. cerealis* was detected in the roots at GS 31 and GS 75. There was a significant interaction between inoculation and season for the amount of *Rhizoctonia* spp. in the roots of the wheat host at GS 31 ($P = 0.049$) and at GS 75 ($P = 0.001$), possibly due to inconsistency in DNA amounts of AG 5 quantified in the two seasons (Table 2). Treatment had no effect on pathogen DNA in soil and in roots at GS 31. At GS 75, DNA in roots of AG 2-1 and

R. cerealis was found at lower concentrations than at the previous growth stage (Table 2). There was a significant interaction between inoculation and treatment (P = 0.025) associated with inconsistency in the effect of treatments in inoculated plots with AG 5. Overall, DNA of AG 2-1 and *R. cerealis* in roots in both seasons was less in plots treated with sedaxane + fludioxonil (Table 2).

At GS 15, there was a significant interaction between inoculation, season, and treatment with higher DNA concentrations of *R. cerealis* and AG 5 in stems in the first season, in contrast to AG 2-1 DNA which accumulated more in the second season (Table 3). Furthermore, the effectiveness of seed treatment to reduce pathogen DNA in stems was inconsistent between species and seasons, for example, fludioxonil reduced *R. cerealis* DNA in the first season but not in the second. At GS 31, the amount of DNA of all pathogens increased in untreated stems compared to GS 15 (Table 3). AG 2-1 and *R. cerealis* DNA in stems was 4 and 12.5-fold higher than AG 5, respectively (P = 0.002) (Table 3). However, there was no effect of seed treatment, and there were no interactions at GS 31. Less DNA accumulated in stems at GS 75 than GS 31, although AG 5 DNA in the first season and *R. cerealis* DNA in the second season were highest from the three pathogens ($P <$ 0.001) (Table 3). Seed treatment had no significant effect on pathogen DNA in stems, and there were no interactions for this growth stage.

Effect of inoculation and seed treatment on *Microdochium* spp. DNA in stems. *M. nivale* and *M. majus* were detected predominantly in 2012/13 at GS 15 (Table 4). There was no significant pathogen or treatment effect for *M. nivale* (Table 4). *M. majus* DNA was lower than DNA of *M. nivale* in plots inoculated with AG 2-1 and *R. cerealis* and was significantly reduced by sedaxane + fludioxonil compared to the untreated.

Yield components. Plant height was reduced significantly by *R. cerealis* and *R. solani* AG 2-1 compared to the not-inoculated plots in both seasons (Table 5). This effect was negated by fludioxonil and sedaxane + fludioxonil treatments in AG 2-1 and *R. cerealis*, however the effect of seed treatments on plants in AG 5 inoculated plots was less consistent (Table 5).

In contrast to plant height, inoculation had no effect on GAI at GS 15 but fludioxonil increased GAI of inoculated plots. Sedaxane + fludioxonil treatment showed the same effect in the first season of experimentation, but in the second experiment this effect was not consistent in AG 5 and AG 2-1- inoculated plots.

Ear partitioning index (EPI) is the fraction of above-ground DM partitioned in the ear. Significant interaction was observed between inoculation and season ($P = 0.048$) (Table S4). Thus in 2012/13 there were only slight differences in EPI between the inoculated and not-inoculated control plots. However, in 2013/14, *R. cerealis* significantly reduced EPI compared to the control (Table S4).

Yield is presented for 2012/13 since in 2013/14 plots were too small (1 x 1 m) to accurately assess field harvest yield on per ha basis. Differences for inoculation were not significant at $P<0.05$, however yields of infected plots with *R. cerealis*, AG2-1, and AG5 were 0.83, 0.44, and 0.22 t ha^{-1}, respectively lower than that of the control (Fig. 4a). *R. cerealis* reduced TGW significantly by 3.6% in both seasons compared to the not-inoculated control (Fig. 4b). There was no significant effect of seed treatment on yield or TGW.

Relationships between disease assessments and pathogen DNA. Regression analyses (R^2) using disease indexes and pathogen DNA revealed that there were no significant correlations between *Rhizoctonia* DNA in roots and root rot (data not shown). There were significant but

306 generally weak to moderate ($R^2 \leq 0.40$) relationships between *Rhizoctonia* spp. DNA in stems and
307 symptoms on the stems (Table S5). The strongest relationship ($R^2 = 0.60$) was between
308 *Rhizoctonia* spp. DNA in stems at GS 15 and stem browning index at GS 15 and between
309 *Rhizoctonia* spp. DNA in stems at GS 15 and sharp eyespot index at GS 75 ($R^2 = 0.45$). Sharp
310 eyespot was negatively related to BFR at GS 31, GS 39, and GS 75. There was also a very weak
311 but significant relationship between stem browning at GS 15 and *Microdochium* DNA in stems at
312 GS 15 (Table S5).

313 **Effect of seed treatment on early disease by *Fusarium* spp., *M. nivale* and *R. cerealis* and**
314 **yield.** To determine the effect of sedaxane + fludioxonil against early effects of other common
315 soil-borne pathogens and final yield, we carried out wheat field experiments in two consecutive
316 seasons using *Fusarium* spp., *M. nivale*, and *R. cerealis* inoculation. Number of plants (m^{-2}) were
317 lowest in the first season of experimentation in plots inoculated with *M. nivale* whilst in the second
318 season in plots inoculated with *R. cerealis* (Fig. 5a). Higher plant numbers were observed in
319 sedaxane + fludioxonil treated plots in both seasons except for *Fusarium* spp. in 2017/18 (Fig.5a).
320 The greatest effect of sedaxane + fludioxonil treatment was seen under *R. cerealis* inoculation in
321 2017, showing 34.3% increase in plant numbers compared to the untreated plots. There was 9%
322 increase under *M. nivale* inoculation. *R. cerealis* caused more severe stem browning disease
323 compared to *Fusarium* spp. or *M. nivale* (Fig. 5b) and treatment reduced disease symptoms in
324 inoculated plots at GS 15 by 43% overall (Fig. 5c). There were no interactions between factors for
325 yield, and yield response to treatment was 0.27 t ha^{-1} ($P = 0.02$) (Fig. 5d).

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Discussion

We show here that *R. cerealis* is capable of causing pre- and post-emergence damping off prior to developing into sharp eyespot, thus causing losses in establishment and in yield of wheat from the combined effects of both seedling and stem-base disease. In contrast, *R. solani* AG 2-1 and AG 5 caused relatively small reductions in emergence, suggesting that these species or the isolates we used in this work are unlikely to cause pre-or post-emergence damping off in field grown wheat. *R. cerealis* has been previously reported to cause damping-off (Hamada et al. 2011a; Parry 1990), but it has not been generally associated with establishment losses. In the second series of field experiments in 2016/17 and 2017/18, we compared the effects of soil-borne *R. cerealis,* identified as the more aggressive *Rhizoctonia* spp., to FSB pathogens such as *Fusarium* spp. or *M. nivale* that are commonly associated with reductions in emergence and establishment (Humphreys et al. 1995) when they are seed-borne. Results using soil-borne inoculum showed that *R. cerealis* followed by *M. nivale* were the main pathogens causing low emergence associated with the greatest reductions in plots in 2016/17. *Microdochium nivale* and *M. majus* confirmed in stems at GS 15 in the untreated plots in 2012/13 were also implicated in reduced emergence, stem browning, and BFR in the first series of field experiments, although it is possible that *M. nivale* and *M. majus* infection may have also been seed-borne. We did not confirm seed load of these species prior to sowing, although seed viability by the seed producer was confirmed. In the second series of experiments, *F. graminearum* and *F. culmorum* failed to cause significant disease from soil-borne inoculum compared to *M. nivale* or *R. cerealis*. It is possible that environmental conditions or the inoculation method used here failed to favour *Fusarium* infection. It is therefore not surprising that the effects of disease or seed treatments were not detected in *Fusarium*-inoculated plots.

There are contrasting results on the pathogenicity and aggressiveness of isolates of AG 2-1 and AG 5 to wheat roots (Roberts and Sivasithamparam 1986; Rush et al. 1994). Throughout the period of these studies there was no significant difference in root rot disease between *R. solani*-inoculated and not-inoculated plots, suggesting that the isolates of AG 2-1 and AG 5 used here were only weakly pathogenic to wheat roots in field conditions. Limitations in accuracy of visual assessments of root rot symptoms can be overcome by quantifying pathogen DNA in roots. Indeed, 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, but no association was found between *Rhizoctonia* DNA in roots and root rot symptoms, indicating that symptoms may be caused by a complex of species in the rhizosphere that cause disease with very similar symptoms (Harris and Moen 1985) or additional damage due to pests. Furthermore, the results here showed significant reductions of root rot symptoms by the application of seed treatments but no consistent effect on individual pathogen DNA in roots corroborating that symptoms may have been also associated with other organisms than AG 2-1, AG 5, or *R. cerealis*.

Stem-base diseases at early growth stages of the host are difficult to distinguish (Brown et al. 2020; Turner et al. 1999; Turner et al. 2001;). Therefore, plants at GS 15 were assessed for indiscriminate stem browning. The highest stem browning consistently occurred in *R. cerealis* inoculated plots in both seasons. In addition, *R. cerealis* DNA was quantified at higher concentrations than AG 2-1 and AG 5 in stems, suggesting that wheat is more susceptible to infection by the adapted *R. cerealis* than by the generalists, 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. *R. 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.

374 This inconsistency in DNA accumulation in soil suggests that differences in environmental
375 conditions play a significant role in the occurrence and severity of stem disease by AG 5. The
376 acute stem browning on seedlings in the not-inoculated control plots in 2012/13 indicated the
377 presence of other pathogens confirmed as *Microdochium* spp. and quantified at relatively high
378 amounts in stems at GS 15 in 2012/13 compared to 2013/14. Since DNA of *Rhizoctonia* spp.
379 rather than of *Microdochium* spp. accounted for 60% of the variation in stem browning at GS 15,
380 we can be confident that *Rhizoctonia* spp. were responsible for the majority of stem symptoms at
381 this early stage. In naturally infected fields, symptoms of sharp eyespot are typically observed
382 following stem extension (Lemańczyk and Kwaśna 2013). However, the inoculation method here
383 delivered inoculum directly next to the sown seed allowing the pathogen to colonize the
384 developing seedling prior to stem extension (GS 31), thus resulting in early sharp eyespot by *R.*
385 *cerealis*. BFR index by the naturally occurring *Microdochium* was significantly lower in *R.*
386 *cerealis* inoculated plots than in AG 2-1, AG 5, or the not-inoculated control throughout the
387 growing season. There were also the weak but significant negative relationships between sharp
388 eyespot and BFR index suggesting competitive interactions between *R. cerealis* and the BFR
389 causing species of *Microdochium*. *R. cerealis* had a competitive advantage due to the higher
390 inoculum density in soil and may have suppressed the naturally occurring *Microdochium* agreeing
391 with observations also made in previous studies by Pettitt et al. (2003).

392 Reduction in plant height, EPI and yield were mostly associated with the effects of *R. cerealis*.
393 Our results suggest that *R. cerealis* causing sharp eyespot imposes significant limitations on source
394 to sink partitioning via biomass reductions resulting in yield loss of 8% similar to the estimated
395 maximum yield losses in Poland of 8-10% in naturally infected commercial wheat fields
396 (Lemańczyk and Kwaśna 2013).

Fludioxonil and sedaxane + fludioxonil increased plant emergence by 47.9 and 50.2%, respectively in 2012/13. Therefore, under high disease pressure caused by a mixture of *Rhizoctonia* and naturally occurring *Microdochium* spp., there was a significant response to both seed treatments. Fludioxonil (Glynn et al. 2007; Glynn et al. 2008) and sedaxane (Zeun et al. 2013) have been previously shown to effectively control *Microdochium* spp. *in vitro* and in field (Jonavičienė et al. 2016). Furthermore, in this study, sedaxane as part of a mixture with fludioxonil, under high disease pressure significantly increased plant counts at GS 39 by 29% compared to the untreated. The effectiveness of the two actives in the seed treatment was confirmed in 2017/18 against emergence losses due to *R. cerealis*, where the observed increase above the control was 35%. This demonstrates an additional benefit of sedaxane in controlling damping off caused by *R. cerealis*, which is in agreement with studies showing high activity of sedaxane on mycelial growth inhibition of *Rhizoctonia* spp. (Da Silva et al. 2017; Zeun et al. 2013). Sedaxane and fludioxonil were also able to reduce root rot on winter wheat at GS 15 in field conditions agreeing with previous report in controlled environment showing similar results on rhizoctonia root rot in maize, corn, and cotton (Zeun et al. 2013). The main period of activity of a seed treatment is generally considered to last 4-6 weeks after sowing, thus the lack of consistency at the later growth stages of 31 and 75 especially in AG 5 inoculated plots was not surprising. Fludioxonil alone was less consistent since its effect was only significant in the 2012/13 experiment. However, the mixture of sedaxane and fludioxonil reduced stem browning and disease index in all experiments. The addition of sedaxane therefore extends the period of effectiveness of seed treatment in controlling early stem-base diseases caused by *Rhizoctonia* spp. and *Microdochium* spp. Quantification of *Microdochium* spp. DNA showed that fludioxonil was consistent in controlling *M. nivale* while no DNA of *M. majus* was detected in sedaxane + fludioxonil treatments in both seasons, suggesting

420 that sedaxane contributed in controlling this species. Seed treated plots also had significantly lower
421 BFR index at GS 31 and GS 39 in our experiments compared to the untreated indicating that
422 fludioxonil controlled BFR up to GS 39. There was no significant effect of seed treatment on
423 *Rhizoctonia* DNA concentrations or sharp eyespot after GS 31, 39, or 75 suggesting that sharp
424 eyespot control required additional stem base fungicide application that should be applied at the
425 beginning of stem extension (GS 30–31) (Nicholson et al. 2002). In the second series of
426 experiments, *F. graminearum* and *F. culmorum* failed to cause significant disease from soil-
427 inoculum compared to *M. nivale* or *R. cerealis*, and we were unable to detect the effects of seed
428 treatments in *Fusarium*-inoculated plots. The inoculum method used here was developed for, and
429 favoured *Rhizoctonia* in contrast to *Fusarium* or even *Microdochium* spp. that have been shown to
430 cause severe seedling blight/foot rot disease from infected seed (Haigh et al. 2009; Ren et al. 2016)
431 or ground surface inoculation simulating left over debris (Jones et al. 2018).

432 Artificially inoculated experiments provide useful information on worst case scenarios for
433 losses due to pathogens applied at high inoculum density, and this approach is appropriate to
434 establish comparative differences and effectiveness of control methods to individual pathogens.
435 Under natural infection, the inoculum density of these pathogens is likely to be lower and for some
436 of them, for example *Fusarium* and *Microdochium* spp. significant threats to the host may arise
437 from other sources of inoculum. This work however focussed on diseases due to these pathogens
438 from soil-borne inoculum and as such is the first report on their comparative effects in wheat.
439 Based on our results, soil-borne disease control in the UK should focus on *R. cerealis* and *M.*
440 *nivale* that reduced wheat emergence and establishment. Fludioxonil and sedaxane were shown as
441 effective seed treatment in reducing damping off and foot rot, thus providing control against more

than one pathogen and disease resulting in a modest but significant response of 4% in yield of wheat.

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562 **Figure legends**

563 **Fig. 1**. Effect of *Rhizoctonia solani* AG 2-1, AG 5, and *R. cerealis* (a) and seed treatment (b) on
564 emergence (%) of wheat seedlings and plant populations (m^{-2}) of winter wheat (cv. Santiago) at
565 GS 39 (c) in 2012/13 and in 2013/14. dpi - days post inoculation, Control - not-inoculated, UT-
566 untreated, F- fludioxonil, S + F- sedaxane and fludioxonil. Error bars indicate SE. * $P < 0.05$; ***:
567 $P < 0.001$. l.s.d. - least significant difference at $P<0.05$.

568 **Fig. 2**. Effect of seed treatment on root rot disease index (DI %) (angular transformed) by
569 *Rhizoctonia solani* AG 2-1, AG 5, or *R. cerealis* at GS 15 (a), GS 31 (b), and GS 75 (c) of winter
570 wheat (cv. Santiago) in 2012/13 and in 2013/14. Control - not-inoculated, UT- untreated, F-
571 fludioxonil, S + F- sedaxane and fludioxonil, l.s.d - least significant difference at $P<0.05$.

572 **Fig. 3**. Effect of inoculation with *Rhizoctonia solani* AG 2-1, AG 5, and *R. cerealis* on sharp
573 eyespot disease index (DI %) (angular transformed) (a) brown foot rot disease index (DI %)
574 (angular transformed) (b), and effect of seed treatment on brown foot rot DI (%) (angular
575 transformed) (c) in winter wheat (cv. Santiago) in 2012/13 and in 2013/14. Control - not-
576 inoculated, F- fludioxonil, S + F- sedaxane and fludioxonil. Error bars indicate SE. * $P < 0.05$;
577 ***: $P < 0.001$.

578 **Fig. 4**. Effect of inoculation with *Rhizoctonia solani* AG 2-1, AG 5, or *R. cerealis* (a) on grain
579 yield (t ha^{-1}) in 2012/13 and thousand grain weight (TGW) (g) (b) of winter wheat (cv. Santiago)
580 in 2012/13 and in 2013/14. Control - not-inoculated. l.s.d - least significant difference at $P<0.05$.

581 **Fig. 5**. Effect of inoculation with *Fusarium graminearium*, *F. culmorum*, *M. nivale*, or *R. cerealis*
582 and treatment with sedaxane and fludioxonil on number of plants (m^{-2}) at 29 dpi (a), stem browning
583 disease index (GS 15) (b and c), and yield (d) of winter wheat (cv. Leeds) in 2016/17 and 2017/18.
584 UT – untreated, S + F - sedaxane and fludioxonil. l.s.d - least significant difference at $P<0.05$.

Supporting information

Supplementary Table 1. Crop protection programme for field experiments in 2012/13 and 2013/14.

Supplementary Table 2. Crop protection programme for field experiments in 2016/17 and 2017/18.

Supplementary Table 3. Primer and probe sequences for *Rhizoctonia* and *Microdochium spp*.

Supplementary Table 4. Effect of inoculation with *Rhizoctonia solani* AG 2-1, AG 5, or *R. cerealis* and seed treatment with sedaxane and/or fludioxonil on ear partitioning index of winter wheat (cv. Santiago) in 2012/13 and 2013/14.

Supplementary Table 5. Summary of significant regressions for stem-base diseases and log_{10} DNA of *Rhizoctonia* spp. in stems of winter wheat (cv. Santiago) in 2012/13 and 2013/14.

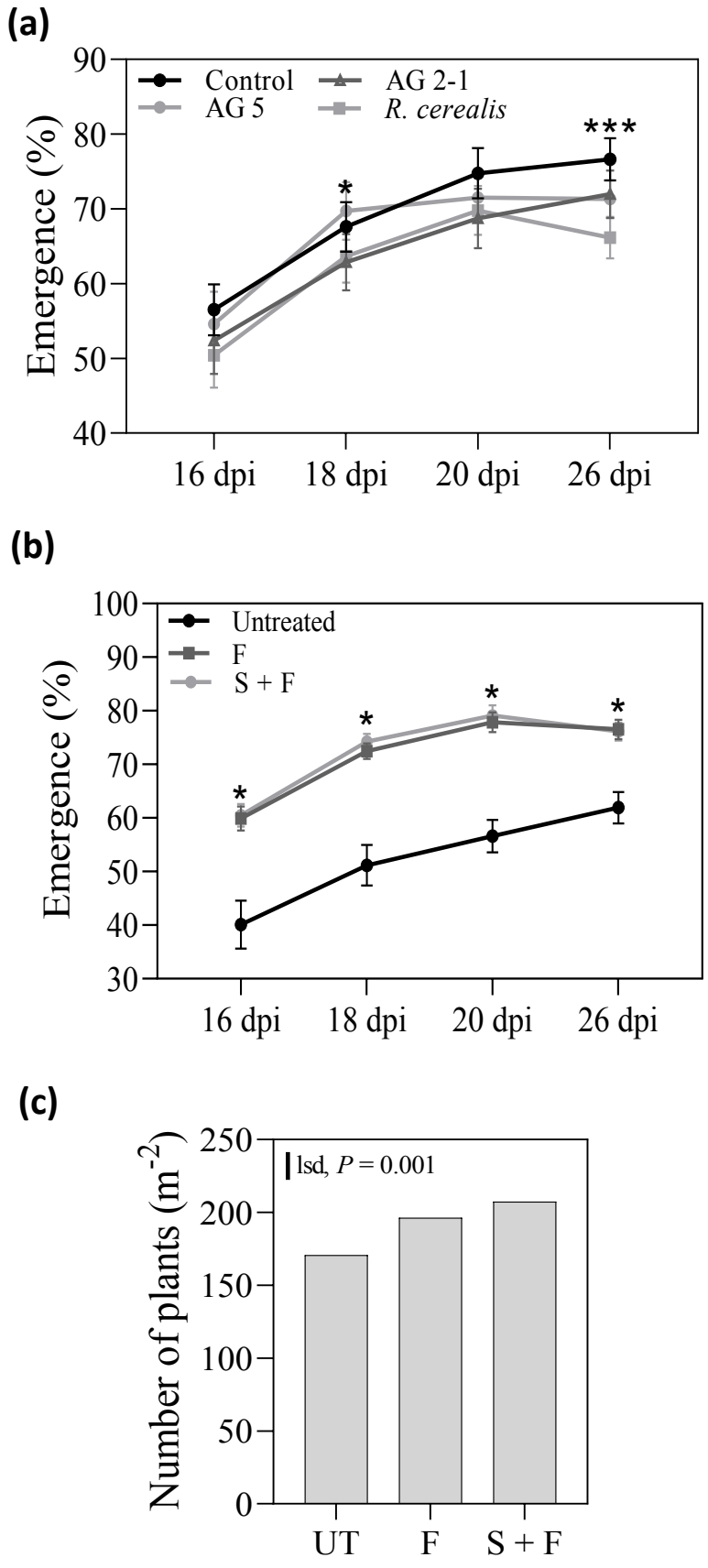

(a)
Control
AG 5
AG 2-1
R. cerealis
Emergence (%)
90
80
70
60
50
40
*
***
16 dpi
18 dpi
20 dpi
26 dpi
(b)
Untreated
F
S + F
Emergence (%)
100
90
80
70
60
50
40
30
*
*
*
*
16 dpi
18 dpi
20 dpi
26 dpi
(c)
lsd, P = 0.001
Number of plants (m-2)
250
200
150
100
50
0
UT
F
S + F

Figure 1.

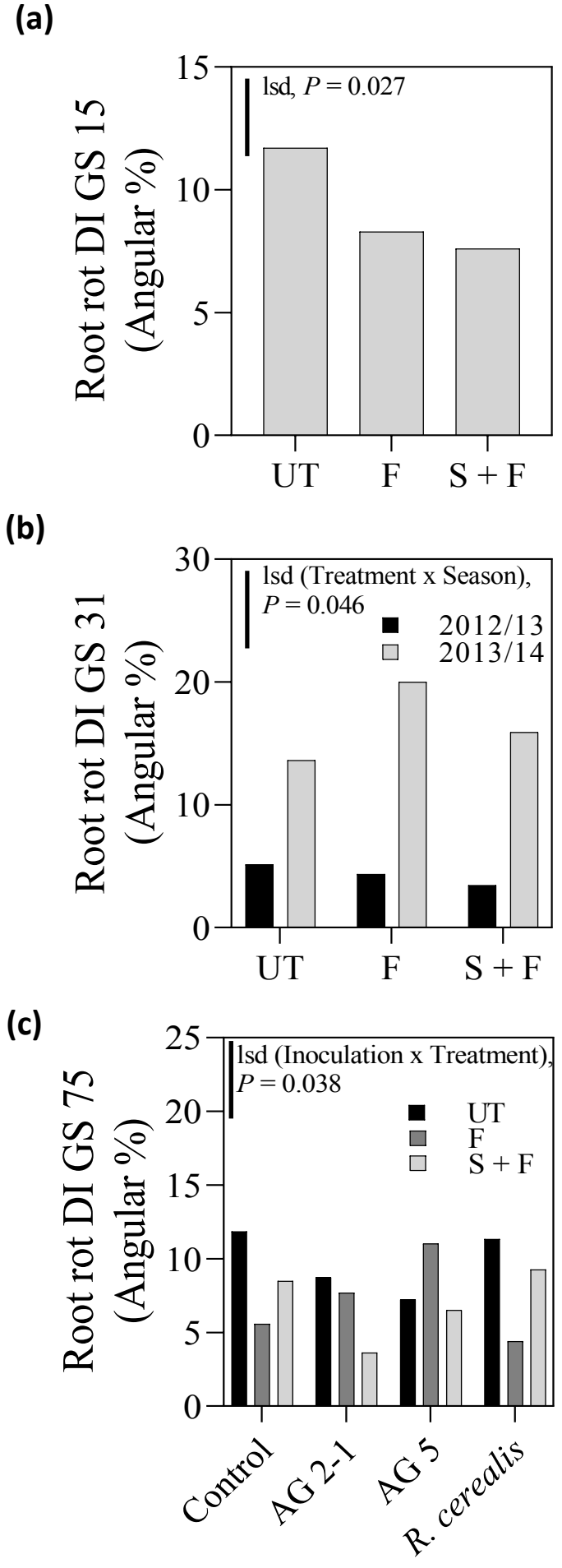

(a)
Root rot DI GS 15 (Angular %)
lsd, P = 0.027
15
10
5
0
UT
F
S + F
(b)
Root rot DI GS 31 (Angular %)
lsd (Treatment x Season), P = 0.046
2012/13
2013/14
30
20
10
0
UT
F
S + F
(c)
Root rot DI GS 75 (Angular %)
lsd (Inoculation x Treatment), P = 0.038
UT
F
S + F
25
20
15
10
5
0
Control
AG 2-1
AG 5
R. cerealis

Figure 2.

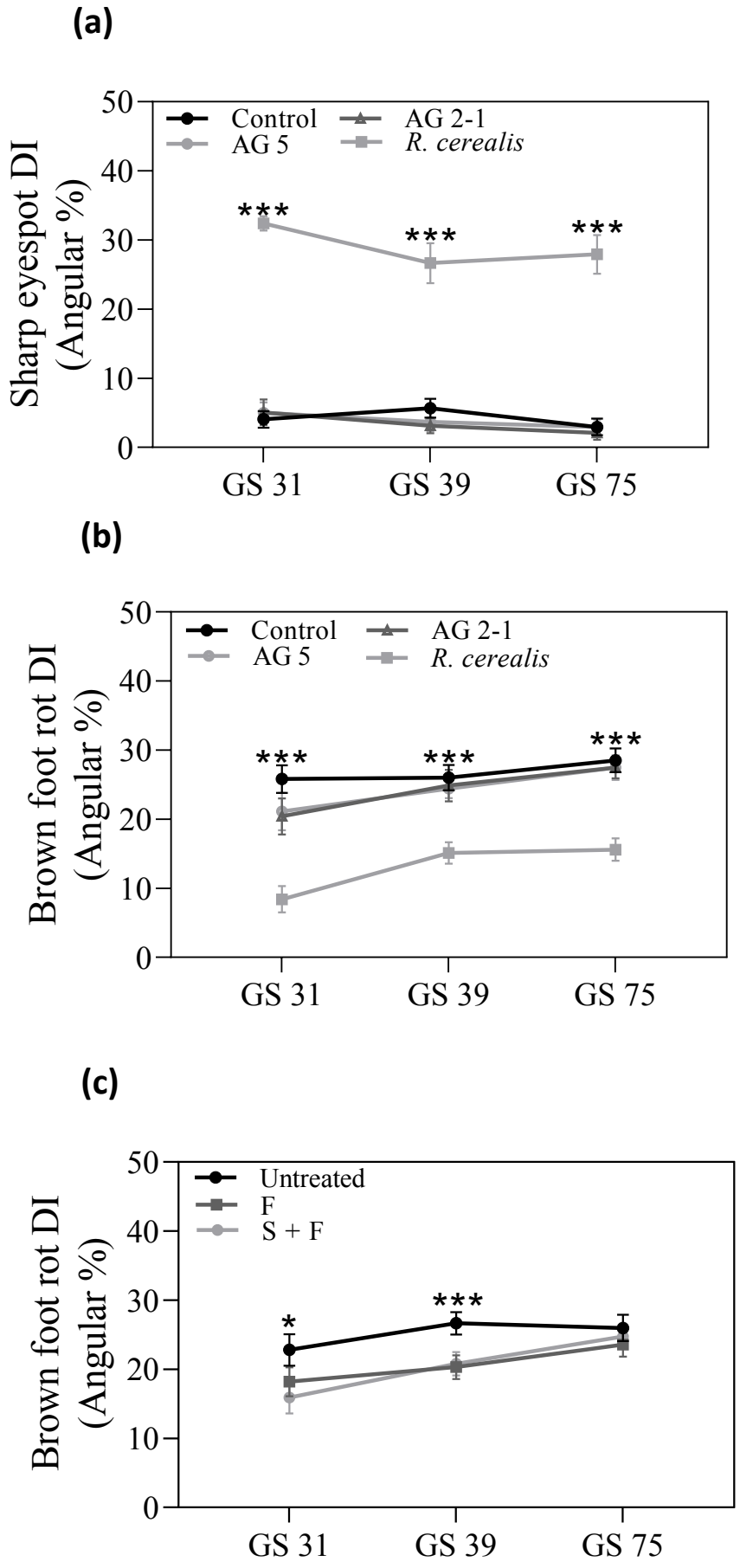
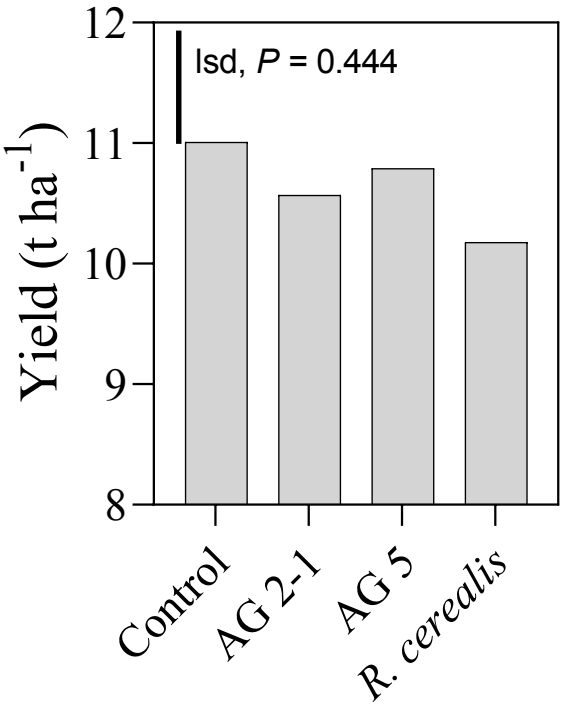

(a)
Sharp eyespot DI (Angular %)
Control
AG 5
AG 2-1
R. cerealis
***
***
***
GS 31
GS 39
GS 75
(b)
Brown foot rot DI (Angular %)
Control
AG 5
AG 2-1
R. cerealis
***
***
***
GS 31
GS 39
GS 75
(c)
Brown foot rot DI (Angular %)
Untreated
F
S + F
*
***
GS 31
GS 39
GS 75

Figure 3.

(a)


12
11
10
9
8
Yield (t ha-1)
lsd, P = 0.444
Control
AG 2-1
AG 5
R. cerealis

(b)

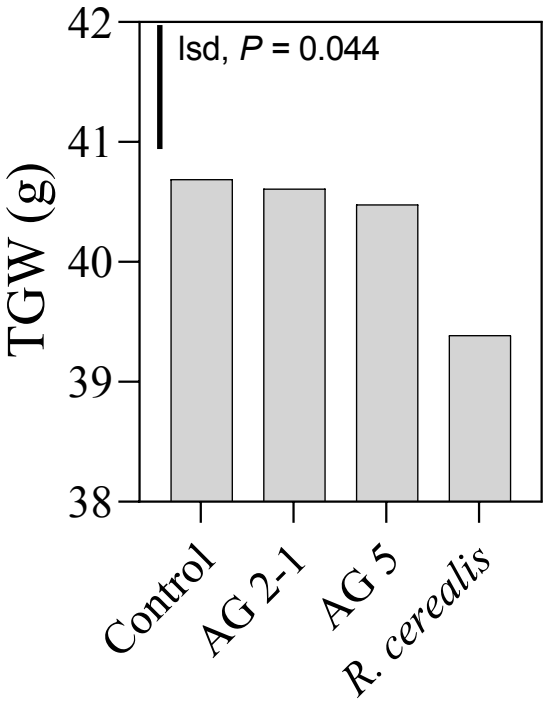

42
41
40
39
38
TGW (g)
lsd, P = 0.044
Control
AG 2-1
AG 5
R. cerealis

Figure 4.

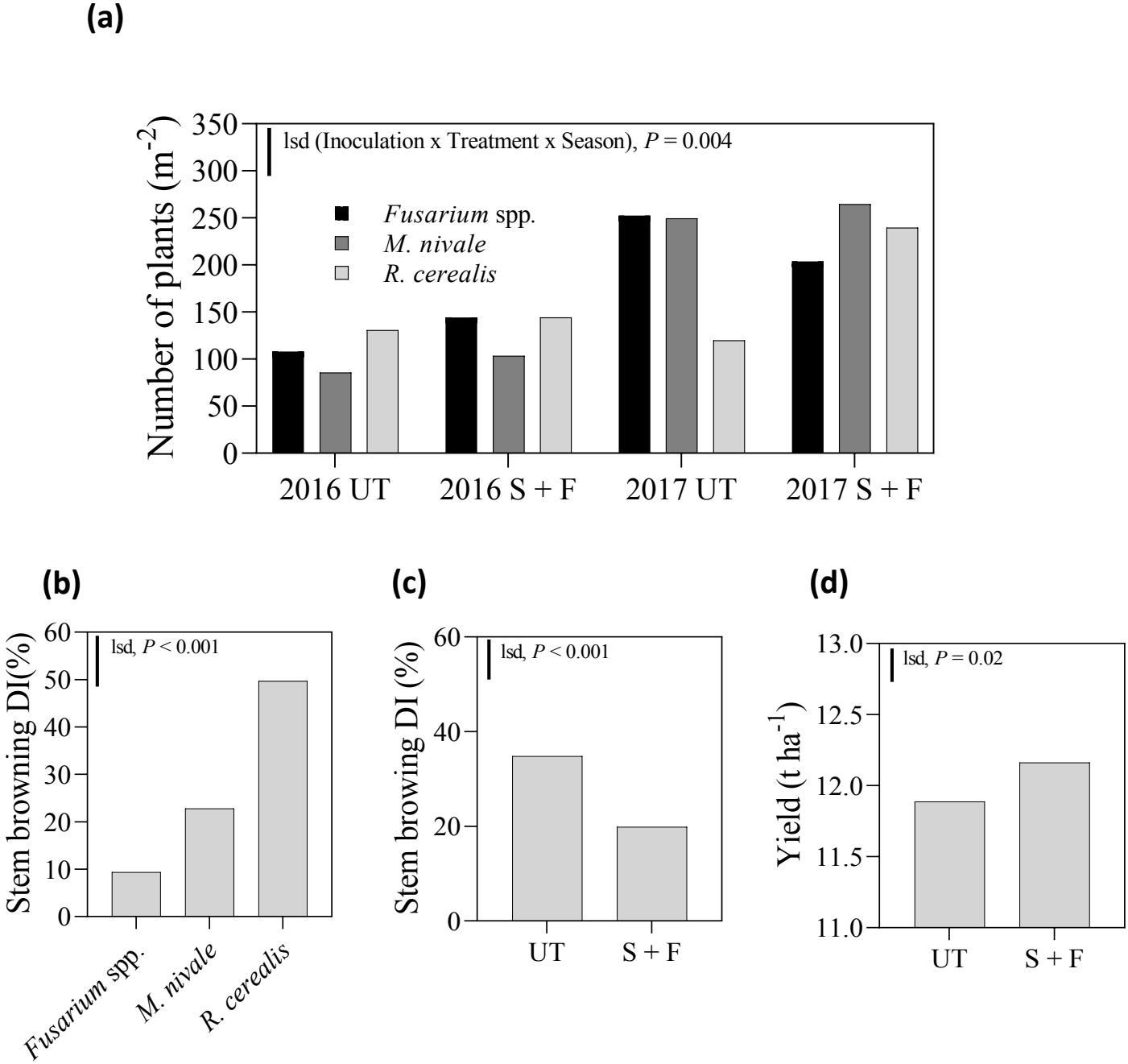

(a)
Number of plants (m-2)
lsd (Inoculation x Treatment x Season), P = 0.004
Fusarium spp.
M. nivale
R. cerealis
2016 UT
2016 S + F
2017 UT
2017 S + F
(b)
Stem browning DI(%)
lsd, P < 0.001
Fusarium spp.
M. nivale
R. cerealis
(c)
Stem browing DI (%)
lsd, P < 0.001
UT
S + F
(d)
Yield (t ha-1)
lsd, P = 0.02
UT
S + F

Figure 5.

Table 1. The effect of inoculation with *Rhizoctonia solani* AG 2-1, AG 5, or *R. cerealis* and seed treatment with sedaxane and/or fludioxonil on stem browning disease index (Angular transformed %) at GS 15 of winter wheat (cv. Santiago) in 2012/13 and 2013/14

	Disease index (%)					
	2012/13			**2013/14**		
Treatment	**UT[b]**	**F[b]**	**S + F[b]**	**UT**	**F**	**S + F**
Control[a]	23	0	0	3.5	5	0
AG 2-1	26	4.6	8.3	9.9	5	2.5
AG 5	37	21	12	6	6	0
R. cerealis	51	46	44	41	42	36

	P-value	LSD[c]
Season	0.003	4.437
Inoculation	<.001	3.822
Treatment	<.001	3.310
Inoculation*Treatment	0.089	6.620
Inoculation*Season	0.002	5.961
Treatment*Season	<.001	5.364
Inoculation*Treatment*Season	0.39	9.682

[a] Control - not-inoculated.

[b] 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}).

[c] LSD – least significant difference of means at $P<0.05$.

Table 2. Effect of inoculation with *Rhizoctonia solani* AG 2-1, AG 5, or *R. cerealis* and seed treatment with sedaxane and/or fludioxonil on DNA of *Rhizoctonia* spp. (pg g^{-1} of soil) in soil at GS 15 and in roots at GS 31 and GS 75 of winter wheat (cv. Santiago) in 2012/13 and 2013/14. Values are log_{10} transformed with back-transformed means in parentheses

	2012/13						2013/14					
Treatment	**UT[a]**		**F[a]**		**S + F[a]**		**UT**		**F**		**S + F**	
GS 15 soil												
AG 2-1	2.47	(295.1)	2.16	(144.5)	2.95	(891.3)	2.35	(223.9)	1.49	(30.9)	-0.41	(0.39)
AG 5	2.15	(141.3)	0.81	(6.46)	1.67	(46.8)	-1.02	(0.10)	0.17	(1.48)	-0.63	(0.23)
R. cerealis	3.62	(4168.7)	1.65	(44.7)	3.44	(2754.2)	3.65	(4466.8)	0.63	(4.27)	2.77	(588.8)
GS31 wheat roots												
AG 2-1	-0.82	(0.151)	-1.06	(0.087)	-0.72	(0.191)	-0.46	(0.347)	-0.95	(0.112)	-2.06	(0.009)
AG 5	-1.68	(0.021)	-1.39	(0.041)	-1.42	(0.038)	-2	(0.010)	-2.69	(0.002)	-1.94	(0.011)
R. cerealis	-1.24	(0.058)	-0.76	(0.174)	-2.19	(0.006)	-0.49	(0.324)	-0.46	(0.347)	-1.19	(0.065)
GS75 wheat roots												
AG 2-1	-2.91	(0.001)	-3.32	(0.0005)	-3.31	(0.0005)	-2.06	(0.009)	-1.80	(0.016)	-3.66	(0.0002)
AG 5	-1.56	(0.028)	-1.28	(0.053)	-0.96	(0.110)	-2.68	(0.002)	-3.18	(0.0007)	-1.65	(0.022)
R. cerealis	-2.09	(0.008)	-1.82	(0.015)	-2.85	(0.001)	-1.11	(0.078)	-1.66	(0.022)	-2.31	(0.005)

	GS15		GS31		GS75	
	P-value	LSD[b]	*P*-value	LSD	*P*-value	LSD
Season	0.091	1.629	0.834	1.221	0.993	0.681
Inoculation	0.013	1.317	0.012	0.563	<.001	0.528
Treatment	0.268	1.317	0.256	0.563	0.364	0.528
Inoculation*Treatment	0.541	2.281	0.281	0.974	0.025	0.914
Inoculation*Season	0.533	2.044	0.049	1.274	0.001	0.837
Treatment*Season	0.575	2.044	0.532	1.274	0.728	0.837
Inoculation*Treatment*Season	0.614	3.318	0.430	1.647	0.206	1.339

[a] UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), and S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}). [b] LSD – least significant difference at $P<0.05$.

Table 3. Effect of inoculation with *Rhizoctonia solani* AG 2-1, AG 5, or *R. cerealis* and seed treatment with sedaxane and/or fludioxonil of DNA of *Rhizoctonia* spp. (pg ng^{-1} of total DNA) in wheat stems at GS 15, GS 31, and GS75 of winter wheat (cv. Santiago) in 2012/13 and 2013/14. Values are log_{10} transformed with back-transformed means in parentheses.

	2012/13						2013/14					
Treatment	**UT[a]**		**F[a]**		**S + F[a]**		**UT**		**F**		**S + F**	
GS 15												
AG 2-1	-2.955	(0.001)	-3.551	(0.0003)	-2.852	(0.001)	-2.485	(0.003)	-2.094	(0.008)	-2.719	(0.002)
AG 5	-1.744	(0.018)	-3.149	(0.001)	-3.584	(0.0003)	-3.691	(0.0002)	-3.164	(0.001)	-4	(0)
R. cerealis	-0.849	(0.142)	-1.111	(0.077)	-0.826	(0.149)	-0.153	(0.073)	-0.812	(0.154)	-1.142	(0.072)
GS31												
AG 2-1	-0.46	(0.35)	-1.54	(0.03)	-0.2	(0.63)	-0.64	(0.23)	-0.57	(0.27)	-1.4	(0.04)
AG 5	-0.86	(0.14)	-0.83	(0.15)	-1.64	(0.02)	-1.78	(0.02)	-2.14	(0.01)	-1.44	(0.04)
R. cerealis	-0.79	(0.16)	0.18	(1.51)	-0.57	(0.27)	0.08	(1.20)	0.23	(1.70)	-0.86	(0.14)
GS75												
AG 2-1	-2.41	(0.004)	-0.89	(0.129)	-1.64	(0.023)	-2.93	(0.001)	-3.48	(0.0003)	-4	(0)
AG 5	-0.52	(0.302)	-0.07	(0.851)	-0.51	(0.309)	-2.3	(0.005)	-2.4	(0.004)	-1.5	(0.032)
R. cerealis	0.17	(0.148)	0.95	(8.910)	0.63	(4.270)	0.22	(1.66)	0.25	(1.78)	-1.13	(0.074)

	GS15		GS31		GS75	
	P-value	LSD[b]	*P*-value	LSD	*P*-value	LSD
Season	0.831	0.459	0.700	1.267	0.003	0.712
Inoculation	<.001	0.366	0.002	0.576	<.001	0.556
Treatment	0.013	0.366	0.642	0.576	0.218	0.556
Inoculation*Treatment	0.141	0.633	0.371	0.998	0.691	0.981
Inoculation*Season	<.001	0.572	0.312	1.32	0.162	0.886
Treatment*Season	0.048	0.572	0.79	1.32	0.114	0.886
Inoculation*Treatment*Season	0.040	0.924	0.106	1.696	0.212	1.43

[a] UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), and S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}). [b] LSD – least significant difference at P<0.05.

Table 4. Effect of inoculation with *Rhizoctonia solani* AG 2-1, AG 5, or *R. cerealis* and seed treatment with sedaxane and/or fludioxonil on DNA of *Microdochium majus* and *M. nivale* (pg ng^{-1} of total DNA) in stems at GS 15 of winter wheat (cv. Santiago) in 2012/13 and 2013/14. Values are log_{10} transformed with back-transformed means in parentheses.

	2012/13						2013/14					
Treatment	**UT[a]**		**F[a]**		**S + F[a]**		**UT**		**F**		**S + F**	
M. nivale												
AG 2-1	-2	(0.01)	-2.94	(0.001)	-3.01	(0.001)	-4	(0)	-4	(0)	-4	(0)
AG 5	-2.56	(0.003)	-2.66	(0.002)	-3.52	(0.0003)	-4	(0)	-4	(0)	-3.3	(0.001)
R. cerealis	-2.07	(0.009)	-3.01	(0.001)	-2.64	(0.002)	-4	(0)	-4	(0)	-4	(0)
M. majus												
AG 2-1	-2.38	(0.004)	-3.79	(0.003)	-4	(0)	-4	(0)	-4	(0)	-4	(0)
AG 5	-2.59	(0.003)	-4	(0)	-4	(0)	-3.12	(0.001)	-4	(0)	-4	(0)
R. cerealis	-2.62	(0.002)	-4	(0)	-4	(0)	-2.58	(0.003)	-4	(0)	-4	(0)

	M. nivale		*M. majus*	
	P-value	LSD[b]	*P*-value	LSD
Season	0.012	0.808	0.118	0.352
Inoculation	0.957	0.657	0.799	0.357
Treatment	0.413	0.657	<.001	0.357
Inoculation*Treatment	0.96	1.138	0.713	0.619
Inoculation*Season	0.641	1.017	0.216	0.504
Treatment*Season	0.268	1.017	0.107	0.504
Inoculation*Treatment*Season	0.809	1.655	0.372	0.875

[a] UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), and S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}). [b] LSD – least significant difference at $P<0.05$.

Table 5. Effect of inoculation with *Rhizoctonia solani* AG 2-1, AG 5, or *R. cerealis* and seed treatment with sedaxane and/or fludioxonil on plant height (mm) and green area (cm^2) at GS 15 of winter wheat (cv. Santiago) in 2012/13 and 2013/14

	Plant height (mm)						Green area (cm^2)					
	2012/13			2013/14			2012/13			2013/14		
Treatment	UT[b]	F[b]	S + F[b]	UT	F	S + F	UT	F	S + F	UT	F	S + F
Control[a]	151.8	162.6	158.7	121.3	124.3	122.7	90.1	120.2	97.9	62.6	59.9	54.2
AG 2-1	147.6	164.2	163.7	110.1	116.4	117.6	96.9	110.1	105.3	49.3	52.8	48
AG 5	152.2	159.1	164.5	118.2	117.8	117.5	100.8	116.1	106.7	66.4	67.8	54.8
R. cerealis	146.1	165.2	156.6	106.2	113.4	118.8	85.7	133.1	109.5	48	56.6	70.1

	P-value	LSD[c]	*P*-value	LSD
Season	<.001	6.281	<.001	17.6
Inoculation	0.045	3.704	0.298	8.83
Treatment	<.001	3.208	<.001	7.65
Inoculation*Treatment	0.24	6.415	0.223	15.3
Inoculation*Season	0.081	7.079	0.605	18.93
Treatment*Season	0.019	6.69	0.012	18.21
Inoculation*Treatment*Season	0.515	10.096	0.546	25.3

[a] Control - not-inoculated. [b] UT – Untreated, F – Fludioxonil (5 g a.i 100 kg^{-1}), and S+F – Sedaxane (10 g a.i 100 kg^{-1}) + Fludioxonil (5 g a.i 100 kg^{-1}). [c] LSD – least significant difference of means at $P<0.05$.

Table S1. Fungicide programme for field experiments in 2012/13 and 2013/14 sown on the 4th October and 24th October in 2012 and 2013.

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

Table S2. Fungicide programme for field experiments in 2016/2017 and 2017/2018 sown on 10th and 19th October in 2016 and 2017.

Timing	Active substance	Product	Rate (l ha^{-1})
T0	Chlorothalonil	Bravo 500	1
T1	Prothiconazole Tebuconazole	Kestrel®	0.75
	Fluxapyroxad	Imtrex®	0.75
	Folpet	Phoenix®	1.5
T1.5	Chlorothalonil	Bravo 500	1
T2	Epoxiconazole Metconazole	Brutus®	1.5
	Fluxapyroxad	Imtrex®	1.5
	Folpet	Phoenix®	1.5
T3	Prothiconazole Tebuconazole	Kestrel®	0.6

Table S3. Primer and probe sequences for *Rhizoctonia* and *Microdochium spp*.

Target	Target region	Primer name	3' modification[a]	Sequence (5' – 3')	Reference
AG 2-1	ITS1	AG 2-1_F		CTTCCTCTTTCATCCCACACA	Budge *et al*. (2009)
		AG 2-1_R		TGAGTAGACAGAGGGTCCAATAACCTA	
		AG 2-1_P	MGB	AAGTAAATTCC CATCTGT	
AG 5	ITS1	AG-5_F		TGATCAGGTGCTCGATGTCGT	Budge *et al*. (2009)
		AG-5_R		CCCTGCAACAGTCGGTT	
		AG-5_P	MGB	CGCAAAGAGGCCGAG	
R. cerealis	ITS1	RcF		AAAGCATCGTCGCCATGAG	Woodhall *et al.* (2017)
		RcR		CTGCCAACACACCGACATGT	
		RcP	FAM-TAMRA	ATAAAATGGAAGGTAGGTGCGGGTGCATAG	
M. nivale	TEF-1α	Mniv1f		TTGGCTTGCACAAACAATACTTTTT	Nielsen *et al*. (2013)
		Mniv1r		AGCACAACAGGCGTGGATAAG	
M. majus	TEF-1α	Mmajus1f		AACCCCTCCCGGGTCAG	Nielsen *et al*. (2013)
		Mmajus1r		GGATAAACGACACTTGAAGACAGAAAA	

[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.

Table S4. Effect of inoculation with *R. solani* AG 2-1, AG 5 or *R. cerealis* and seed treatment with sedaxane and/or fludioxonil on ear partitioning index of winter wheat (cv. Santiago) in 2012/13 and 2013/14.

	2012/13	2013/14	Mean
Control	0.362	0.360	0.361
AG 2-1	0.355	0.373	0.362
AG 5	0.353	0.350	0.352
R. cerealis	0.355	0.327	0.343
	P-value	l.s.d.	
Season	0.902	0.054	
Inoculation	0.060	0.014	
Treatment	0.565	0.014	
Inoculation*Treatment	0.383	0.028	
Inoculation*Season	0.048	0.020	
Treatment*Season	0.567	0.056	
Inoculation*Treatment*Season	0.063	0.066	

Control - not-inoculated. l.s.d. – least significant difference of means at $P<0.05$.

Table S5. Summary of significant regressions for stem-base diseases and *log*$_{10}$ DNA of *Rhizoctonia* spp. in stems of winter wheat (cv. Santiago) in 2012/13 and 2013/14.

Response variate (y)	Independent variate (x)	Equation	R^2	*P* value
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	Brown foot rot index GS 31	y = 21.57 – 0.53x	0.20	<.001
Sharp eyespot index at GS 39	Brown foot rot index GS 39	y = 24.86 – 0.67x	0.23	<.001
Sharp eyespot index at GS 75	Brown foot rot index GS 75	y = 27.4 – 0.74x	0.26	<.001