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Population dynamics of *Rhizoctonia*, *Oculimacula*, and *Microdochium* species in soil, roots, and stems of English wheat crops

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Abstract

This study aimed to elucidate the population dynamics of *Rhizoctonia*, *Oculimacula*, and *Microdochium* species, causing the stem base disease complex of sharp eyespot, eyespot, and brown foot rot in cereals. Pathogen DNA in soil, roots, and stem fractions, and disease expression were quantified in 102 English wheat fields in two seasons. Weather data for each site was collected to determine patterns that correlate with assessed diseases. *Oculimacula* spp. (66%) and *R. solani* AG 2-1 (63%) were most frequently detected in soil, followed by *R. cerealis* (54%) and *Microdochium* spp. (33%). *Oculimacula* spp. (89%) and *R. cerealis* (56%) predominated on roots and soil but were not associated with root rot symptoms, suggesting that these species used soil and roots for survival and as inoculum source. *M. nivale* was more frequently detected than *M. majus* on stems up to GS 21–30 and co-occurred on plant samples with *O. acuformis*. *O. yallundae* had higher DNA concentration than *O. acuformis* at the lower 5 cm basal region at GS 37–45. *R. cerealis* predominated in the upper 15 cm above the base beyond stem extension. Brown foot rot by *Microdochium* spp. was favoured by cool and wet autumns/winters and dominated in English wheat. Eyespot and sharp eyespot disease index by *Oculimacula* spp. and *R. cerealis*, respectively, correlated with wet/humid springs and summers. Results suggested that stem base pathogens generally coexisted; however, their abundance in time and space was influenced by favourable weather patterns and host development, with niche differentiation after stem extension.

KEYWORDS

Microdochium spp., *Oculimacula* spp., *Rhizoctonia* spp., soil, stem base diseases, wheat

1 | INTRODUCTION

The stem base disease (SBD) complex of wheat consists of three components: eyespot, brown foot rot (BFR), and sharp eyespot (Nicholson & Turner, 2000). Eyespot is caused by either *Oculimacula acuformis* or *O. yallundae*, BFR is principally associated with *Microdochium nivale* or *M. majus* (Turner et al., 2002), and sharp eyespot is caused by *Rhizoctonia cerealis* (Turner et al., 1999). The

populations of these species in soil and in planta can change due to selection pressure from environmental (e.g., rainfall, temperature) and agronomic (e.g., fungicides) factors (Turner et al., 2002).

The last studies in the UK, more than 20 years ago (Nicholson & Turner, 2000; Turner et al., 1999), showed that *O. acuformis* was the main pathogen causing eyespot. However, since then, the introduction and use of the fungicide cyprodinil with increased effectiveness against *O. acuformis* (Parnell et al., 2008) is expected to have caused changes in the eyespot population in favour of *O. yallundae*. Turner et al. (1999) also reported *M. majus* as the major BFR pathogen, and although seasonal variations have been known to occur (Nicholson & Turner, 2000), the pathogen predominance in *Microdochium* populations remains unclear. The distribution, relative abundance, and pathogenic activity of *R. cerealis* worldwide is presumed to be increasing due to climate change and changes in agronomic practices such as earlier sowing dates (Hamada et al., 2011); however, the incidence of this pathogen in UK wheat is unknown. Furthermore, limited information exists on the populations of other *Rhizoctonia* spp. likely to cause disease in English wheat crops. *R. solani* is a soilborne pathogen species complex of 13 anastomosis groups (AGs) (Carling et al., 2002) causing diseases in a broad range of crops including wheat (Ogoshi, 1996). Symptoms caused by *R. solani* include pre- and post-emergence damping off, root rot, foliar blight, and stem rots. *R. solani* AGs 2-1, 4, 5, 8, and 11 are known to cause disease on the roots or stems of wheat (Woodhall et al., 2012a). Isolates of *R. solani* attack young roots of their hosts (Harris & Moen, 1985), and the most recent study has shown that AG 2-1 is capable of reducing the number of primary roots, root volume, and root surface area on 6-day-old wheat seedlings (Sturrock et al., 2015).

Environmental factors such as meteorological conditions influence the development of fungal pathogens in soil and in planta and consequently alter root and stem base disease development and severity. For example, moisture absorbed by infected straw, on which the pathogen resides, is required for sporulation of ascospores and conidia (Rowe & Powelson, 1973) for eyespot infection to occur. Regular rainfall events and cooler temperatures also favour the development of *M. nivale* and *M. majus* in planta (Xu et al., 2008), whilst ambient temperature increases from 16 to 28 °C reduce the disease index for wheat seedlings infected with *R. cerealis* and halts disease progression (Burpee et al., 1980). Information on the relative abundance and niche of species within the pathogen complex during the physiological development of the host informs us on the risk of yield loss associated with their stem base diseases. Such information can be used to improve local management decisions, because the effectiveness of control methods differ for individual species within the complex.

The aim of this study was to define the population dynamics of *Oculimacula*, *Microdochium*, and *Rhizoctonia* spp. as the principal pathogens causing SBD in soil and on stems of naturally infected English winter wheat crops during two seasons. The individual objectives were to (a) determine the incidence and severity of diseases on the roots and stems of winter wheat crops; (b) quantify targeted species of *Rhizoctonia* spp., *Oculimacula* spp., and *Microdochium* spp. at different developmental stages of the crop using quantitative real-time PCR (qPCR); and (c) identify meteorological factors that influence disease severity and pathogen DNA accumulation in soil and in planta.

2 | MATERIALS AND METHODS

2.1 | Soil and plant sampling

The present study sampled 102 commercial winter wheat fields on 22 farms in 20 counties across England. Farms were chosen to give a representative sample of winter wheat fields in the north, Midlands, and south of England. On each farm the fields were randomly identified except for the criteria that wheat predominated in the rotation. This resulted in soil and plant samples being collected from a wide range of soil textures, environmental conditions, and production systems in England. During the 2011/12 and 2012/13 seasons, 52 and 50 fields were sampled, respectively. Farms sampled in 2011/12 were retained for the following season but new fields were identified where wheat came into the rotation.

In each season, soil samples from each field were collected prior to the sowing of the crop (presowing). Soil and plant samples were collected at growth stage (GS) 21–31, GS 37–45, and GS 65–75 (Zadoks et al., 1974). At each sampling period, samples were removed from the same 1 ha area of the field, allowing changes in disease progression and pathogen populations to be observed in the same area. The area sampled was chosen away from the headland, in the mid-field area of the crop. This study used a systematic pattern of collecting soil and plants as this provides a better estimate of changes of populations over random sampling methods (Campbell & Neher, 1994). Samples were removed along 5 × 100 m parallel transects, spaced at 20 m intervals. Along each transect six soil cores and six plants were removed, totalling 36 soil cores and 36 plants from each field. Soil cores were taken to a depth of 10–15 cm. Plants retaining all tillers were harvested along with the top 10–15 cm of the root system. Plant material was placed in separate polythene bags and stored at 4–5 °C with collected soils as a separate sample within 12 hr of removal. Disease assessments were made within 36 hr of sample collection. Following disease assessments, individual plant fractions (tissues) were finely chopped and stored at −25 °C until DNA extraction was performed.

2.2 | Disease assessments

2.2.1 | Root rot

Prior to visual assessment, plant roots and stems were vigorously washed in water to remove all soil. Roots were visually assessed for root rot symptoms that can be caused by *R. solani* using the classification previously described by Strausbaugh et al. (2004). Incidence

was recorded as a percentage of the assessed plants showing symptoms of the disease. A disease index (DI) was calculated using Equation 1, where v = number of plants with <10% lesions on roots, w = number of plants with 10%–33% lesions, x = number of plants with 34%–66% lesions, y = number of plants with >66% lesions, and z = total number of plants assessed.

$$DI = \left\{ \left[v + (w \times 2) + (x \times 3) + (y \times 4) \right] / \left[z \times 4 \right] \right\} \times 100 \quad (1)$$

2.2.2 | Eyespot, sharp eyespot, and BFR

Classification of eyespot, sharp eyespot, and BFR severity was based on the key described by Scott and Hollins (1974). Roots were visually assessed for root rot symptoms and stems were visually assessed for eyespot, BFR, and sharp eyespot symptoms. Stems were assessed at GS 21–31 and GS 37–45 on all tillers, and at GS 65–75 on the main stem of each plant. All tillers were assessed at early growth stages to try to increase the detection of these diseases, which can be difficult to identify early in the season.

Incidence was recorded as a percentage of the assessed plants showing symptoms of the disease. Disease index (DI) was calculated using Equation 2, where v = number of plants with slight symptoms, w = number of plants with moderate symptoms, x = number of plants with severe symptoms, and y = total number of plants assessed.

$$DI = \left\{ \left[v + (w \times 2) + (x \times 3) \right] / \left[y \times 3 \right] \right\} \times 100 \quad (2)$$

2.3 | DNA extraction

DNA was extracted from 250 g of soil using the method developed by Woodhall et al. (2012b). Thirty-six soil cores removed from each field were first homogenized and a representative 250 g sample was used for DNA extraction.

DNA from the plant samples was extracted from three fractions: (a) roots; (b) stem bases (5 cm of the stem basal region) at GS 21–31, GS 37–45, and GS 65–75; and (c) stems (15 cm above the top 5 cm basal region) only at GS 37–45 and GS 65–75. Assessed frozen samples were freeze dried, homogenized, and milled using a centrifugal mill (ZM 200; Retsch) with a 1 mm screen. Between samples the mill was thoroughly cleaned to avoid cross-contamination. Samples were stored at −20 °C until DNA extractions were performed. DNA extraction followed the protocol described by Ray et al. (2004).

2.4 | Targeted qPCR assays

qPCR assays were performed on DNA extracted from soil, roots, stem bases, and stems. Targeted *Rhizoctonia* spp. were *R. solani* AG 2-1, AG 3 (PT), AG 4 (II, II), AG 5, AG 8, and AG 9 (Budge et al., 2009; Woodhall et al., 2013), and *R. cerealis* (Woodhall et al., 2017). qPCR assays also targeted *O. yallundae*, *O. acuformis* (Walsh et al., 2005), *M. nivale*, and *M. majus* (Nielsen et al., 2013). Probes and primers are shown in Tables S1, S2 and S3.

2.5 | Quantification of *Rhizoctonia* spp.

The amplification and quantification of *Rhizoctonia* spp. in soil or plant material were performed using qPCR (TaqMan) assays in 96-well plates using the Applied Biosystems 7500 real-time PCR system. Cycling conditions and reagent volumes are presented in Table S4. Environmental Master Mix 2.0 (Applied Biosystems) for real-time PCR assays was used to target *Rhizoctonia* spp. in DNA extracts originating from soil samples.

2.6 | Analysis of qPCR (TaqMan) assays

The cycle threshold (C_t) value for each reaction was assessed using the Sequence Detection Software's default threshold setting of 0.2 ΔRn (fluorescence) units. Each sample was tested in two replicates and an average C_t was taken. Target DNA in soil samples was quantified by including five DNA standards on each PCR run. The total DNA of standards was first quantified using a NanoDrop spectrophotometer and adjusted to known concentration from the appropriate culture to produce a dilution series of four 10-fold dilutions. Target DNA was then determined by linear regression on C_t. Detection limit for all pathogens in soil assays was 10^{-4} pg/g of soil.

2.7 | Quantification of *Oculimacula* spp. and *Microdochium* spp.

The amplification and quantification of *Oculimacula* spp. and *Microdochium* spp. in soil or plant material was performed using qPCR assays in 96-well plates on the CFX96 Touch real-time PCR Detection System (Bio-Rad). Cycling conditions and reagent volumes are presented in Table S4.

DNA from known isolates of each species were included in each assay to make standard curves (10–10^{-6} ng/μl) and target DNA was then quantified by linear regression. All qPCR assays contained negative controls of nuclease-free water. Quantification of *Rhizoctonia* spp., *Oculimacula* spp., and *Microdochium* spp. DNA in soil samples was expressed as picograms of DNA per gram of soil (pg/g of soil) and for plant material as picograms per nanogram of total DNA (pg/ng). The detection limit of all pathogens in plant assays was 10^{-4} pg/ng of total DNA.

2.8 | Meteorological data

Meteorological records for mean daily maximum ($Temp_{max}$), minimum ($Temp_{min}$) temperature (°C), total rainfall (mm), and mean daily relative humidity (%) were obtained from the ECMWF (European

Centre for Medium-Range Weather Forecasts). The ECMWF uses a numerical model system combined with observational data for reanalysis of the past weather. Therefore, the meteorological data presented are estimates based on the ECMWF model output for the time period of this study. The area data covers a grid with a spatial resolution of 0.25° latitude/longitude (grid cell size c.27.5 km). The latitude/longitude of each field was obtained using global positioning system (GPS) coordinates of the area sampled within each field using Google Earth (Google Earth, 2011). Field coordinates were matched to the nearest grid cell in the ECMWF model to obtain records for that particular field. Records of the meteorological factors for each field were compiled from the date the first samples were collected (presowing) until the date the last samples were collected (GS 65–75) in each survey. Table S5 presents the means for each meteorological variable. Data from the meteorological records were split into three intervals based on the period of time between sampling of each field. These intervals were linked to the seasons during the period of study. Therefore, the interval between presowing to GS 21–31 is the autumn/winter period, the interval between GS 21–31 and GS 37–45 is spring, and between GS 37–45 and GS 65–75 is summer.

2.9 | Statistical analysis

GenStat v. 16.1 (VSN International Ltd.) was used for all statistical analysis. Quantified DNA of *Rhizoctonia* spp., *Oculimacula* spp., and *Microdochium* spp. is presented as means with 95% confidence intervals for each species in soil and plants. Correlation analysis was performed between diseases or pathogen DNA in soil or plants with their associated meteorological interval. Correlations are presented with Pearson coefficients (r) at $p \leq .05$. Further associations were explored between diseases, pathogens, and meteorological variables at each sampling period in the soil, roots, stem bases, and stems using principal components analysis (PCA). The results of the PCA are graphically drawn as biplots, showing the variables as vectors. Vectors that follow acute angles are associated while vectors that are perpendicular have no association, and vectors in opposing directions have negative associations. Missing values were removed and pathogen DNA concentrations were $\log_{10}$ transformed before correlation coefficients and PCA were performed. PCA used a correlation matrix because variables were on different scales. Residuals and individual PC scores are shown in Table S6. PC1 and PC2 captured most of the variation and were used to visualize the data.

3 | RESULTS

3.1 | Incidence and severity of root rot and stem base diseases

Disease incidence as a percentage of fields sampled and as a percentage of plants sampled per field and disease index (DI) for BFR, eyespot, sharp eyespot, and root rot are shown in Table 1. Root rot was present in >90% of crops at GS 21–31, declining as plants matured in both seasons. BFR incidence ranged between 92% and100% of crops and was the dominant SBD apart from GS 37–45 in 2011/12, when eyespot predominated. Eyespot was more prevalent in 2011/12, occurring in 100% and 98% of crops at GS 37–45 and GS 65–75, respectively. Sharp eyespot also occurred more frequently in 2011/12 than in 2012/13, and was identified in 87% and 90% of crops at GS 37–45 and GS 65–75, respectively.

Disease incidence as a percentage of plants sampled per field identified root rot on around 27% of plants at GS 21–31 (Table 1). Overall, BFR occurred most commonly, found on 24%–46% of plants across all growth stages. Eyespot was highly prevalent in 2011/12

TABLE 1 Mean disease incidence (I, %) and disease index (DI) for root rot, eyespot, brown foot rot, and sharp eyespot on English winter wheat fields and plants collected at growth stage (GS) 21–31, GS 37–45, and GS 65–75

		GS 21–31		GS 37–45		GS 65–75	
Disease	**Measure**	**2011/12**	**2012/13**	**2011/12**	**2012/13**	**2011/12**	**2012/13**
Root rot	I (fields)	100.0	94.0	94.0	82.0	52.0	58.0
	I (plants)	28.0	26.0	24.0	14.0	7.0	8.0
	DI	14.6	13.0	11.8	6.8	3.7	4.2
Eyespot	I (fields)	62.0	30.0	100.0	88.0	98.0	74.0
	I (plants)	13.0	3.0	61.0	16.0	49.0	15.0
	DI	8.4	2.2	40.8	10.8	29.2	11.7
Brown foot rot	I (fields)	92.0	94.0	100.0	100.0	100.0	100.0
	I (plants)	24.0	39.0	37.0	46.0	41.0	46.0
	DI	14.1	23.7	27.8	30.7	30.2	28.3
Sharp eyespot	I (fields)	17.0	50.0	87.0	64.0	90.0	52.0
	I (plants)	2.0	8.0	20.0	10.0	26.0	6.0
	DI	1.1	4.9	13.4	7.1	18.1	5.0

Note: 2011/12 $n = 52$, total 1,872 plants disease assessed per growth stage. 2012/13 $n = 50$, total 1,800 plants disease assessed per growth stage.

with incidence peaking at GS 37–45 of 61% and declining to 49% on plants at GS 65–75. In contrast, eyespot in 2012/13 was identified at a relatively low incidence (<20%) although the trend of disease incidence remained the same over the growing season. Sharp eyespot incidence on plants was also higher in 2011/12 than 2012/13, with 20% and 26% of plants infected at GS 37–45 and GS 65–75, respectively.

Disease index (DI) is a measure of disease intensity (calculated from incidence and severity values). Root rot DI was highest at GS 21–31 (c.14%) in both seasons but then declined by GS 65–75 (c.4%) (Table 1). BFR generally had the highest DI of the SBDs (range 14.1%–30.7%). Eyespot was most severe at GS 37–45 (40.8%) in 2011/12, whilst sharp eyespot had the lowest DI of the SBDs and in 2012/13 remained low throughout the season (<7.5%).

3.2 | Pathogen quantification in soil and roots

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

DNA of *R. solani* AG 2-1 was highest in soil samples followed by DNA of *O. yallundae*, *R. cerealis*, *O. acuformis*, *M. nivale*, and *M. majus* (Table 2). The overall trend in both seasons showed *R. solani* AG 2-1 DNA generally declined over the growing season in both years. *R. cerealis* DNA in soil was also higher in 2011/12 than 2012/13. In both seasons, *R. cerealis* DNA in soil showed a similar trend, increasing over the autumn/winter period then decreasing towards summer (GS 65–75). DNA concentrations of *O. yallundae* in soil samples were always higher than *O. acuformis*. There were no clear patterns of development of either *Oculimacula* spp. in soil. In 2011/12, *M. nivale* DNA was higher than *M. majus*, but the reverse was shown in 2012/13. This study also detected *R. solani* AG 5 (5%) and AG 8 (2%) in presowing soil samples in 2011/12 (data not shown). In presowing samples in 2012/13, *R. solani* AG 8 was again detected in 2% of soil samples. *R. solani* AG 5 DNA concentrations ranged from 2.12 to 12.22 pg/g of soil and AG 8 was from 4.71 to 6.81 pg/g of soil. Due to the low incidence, no further PCR assays were performed for these species. In addition, no *R. solani* AG 3 (PT), AG 4 (II, III), or AG 9 were detected in presowing samples in 2011/12.

On average, *O. yallundae* (90%) and *O. acuformis* (88%) were the most frequent species detected in root samples (Table 3). These were followed by *R. cerealis* (56%) and *R. solani* AG 2-1 (52%). The species with the highest DNA concentrations on the roots of wheat was *O. yallundae*. This was followed by *O. acuformis*, *R. cerealis* and *R. solani* AG 2-1, which were just detectable. DNA of all quantified species increased from GS 21–31 to GS 65–75 in both seasons.

3.3 | Pathogen quantification in stem bases and upper stems

Oculimacula spp. and *Microdochium* spp. were the most frequently detected species, present in >90% of samples at each growth stage (Table 4). Less frequently found were *R. cerealis* (74%) and *R. solani* AG 2-1 (39%). The incidence of *R. cerealis* was, on average, higher in 2011/12 (95%) samples than 2012/13 (53%). In both seasons *O. yallundae* was the predominant species in stem bases, followed by *O. acuformis*, *M. nivale*, *M. majus*, and *R. cerealis* (Table 4). Only trace amounts of *R. solani* AG 2-1 were quantified in stem bases. DNA of *Oculimacula* spp. increased 27- and 52- fold for *O. acuformis* and *O. yallundae*, respectively, between GS 37–45 and GS 65–75.

Oculimacula spp. and *R. cerealis* were widespread on the higher stem region of 15 cm above the base of wheat at GS 37–45 and GS 65–75, generally occurring in >75% of samples (Table 5). However, *R. cerealis* in 2012/13 was rarely detected at GS 37–45, yet by GS 65–75 was present in 78% of samples. Based on quantified DNA, overall, the dominant species was *R. cerealis* (Table 5). In both seasons, the amount of *R. cerealis* DNA increased in stem samples, most notably in 2012/13 when DNA increased >1,500-fold. DNA concentrations of *O. acuformis* on stems increased 32-fold in 2011/12, but in the following season failed to develop. The amount of *O. yallundae* DNA on stems increased in both seasons; this was most notable in 2011/12 when the pathogen increased 10-fold.

3.4 | Correlation coefficients (*r*) between root rot, SBDs, and meteorological variables

Meteorological data were split into three intervals based on the period of time between sampling of each field. These intervals were linked to the seasons during the period of study (Table 6). Meteorological data showed that throughout 2011/12 the mean $Temp_{max}$ and $Temp_{min}$ were higher than in 2012/13 (Table S5). Mean total rainfall in 2012/13 over the autumn/winter period doubled that in 2011/12 (Table 6). In contrast, in 2011/12 rainfall in the spring and summer period was higher than in 2012/13. There was a considerable difference in rainfall in the summer period; in 2011/12 there was 146 mm more rainfall than in 2012/13. Mean relative humidity over the autumn/winter period was slightly higher in 2012/13 than 2011/12. However, in 2011/12 from GS 37–45 to GS 65–75 relative humidity was 3.4% and 4.7% higher in spring and summer than at the same periods in 2012/13.

TABLE 2 Pathogen DNA in soil samples from English winter wheat fields collected at presowing, growth stage (GS) 21–31, GS 37–45, and GS 65–75 in 2011/12 and 2012/13

	Presowing		GS 21–31		GS 37–45		GS 65–75	
Pathogen	**2011/12**	**2012/13**	**2011/12**	**2012/13**	**2011/12**	**2012/13**	**2011/12**	**2012/13**
Fungal DNA (pg/g of soil) mean ± 95% CI								
AG2-1	427.30 ± 326.10	376.60 ± 245.90	943.00 ± 685.30	200.30 ± 124.90	404.60 ± 378.30	230.90 ± 151.52	187.80 ± 102.90	125.40 ± 97.23
Rc	103.20 ± 50.45	30.50 ± 23.90	139.80 ± 80.61	89.24 ± 47.10	73.16 ± 44.63	22.74 ± 14.29	130.14 ± 73.38	53.28 ± 37.77
Oa	2.83 ± 2.38	28.66 ± 44.54	0.76 ± 0.38	14.62 ± 16.49	0.39 ± 0.28	23.53 ± 30.90	0.55 ± 0.26	11.57 ± 12.21
Oy	32.90 ± 31.97	135.90 ± 140.90	215.90 ± 406.20	85.93 ± 158.8	41.61 ± 64.08	33.83 ± 41.67	18.20 ± 16.20	126.80 ± 239.60
Mn	0.28 ± 0.29	4.94 ± 4.56	12.87 ± 10.00	1.96 ± 0.99	2.47 ± 2.68	3.45 ± 1.66	4.32 ± 2.38	3.87 ± 2.60
Mm	0.18 ± 0.18	5.24 ± 4.13	0.07 ± 0.10	2.32 ± 1.14	0.61 ± 0.87	2.08 ± 1.04	1.23 ± 1.57	8.87 ± 3.35
Incidence (%)								
AG2-1	67	67	56	64	58	67	62	65
Rc	62	50	58	54	52	44	60	54
Oa	67	67	81	74	69	71	80	80
Oy	90	51	75	68	63	67	64	68
Mn	10	22	62	45	29	44	44	44
Mm	10	33	4	45	4	48	20	62

Note: R. solani (AG2-1),*R. cerealis* (Rc), *O. acuformis* (Oa), *O. yallundae* (Oy), *M. nivale* (Mn), *M. majus* (Mm). Presowing 2011/12 $n = 42$, 2012/13 $n = 46$; GS 21–31 onwards 2011/12 $n = 52$, 2012/13 $n = 50$.

TABLE 3 Pathogen DNA in roots of English winter wheat plants collected at growth stage (GS) 21–31, GS 37–45, and GS 65–75 in 2011/12 and 2012/13

	GS 21–31		GS 37–45		GS 65–75	
Pathogen	**2011/12**	**2012/13**	**2011/12**	**2012/13**	**2011/12**	**2012/13**
Fungal DNA (pg/ng of total DNA) mean ± 95% CI						
AG2-1	0.001 ± 0.001	0.00001 ± 0.00001	0.0002 ± 0.0002	0.001 ± 0.001	0.004 ± 0.006	0.001 ± 0.001
Rc	0.07 ± 0.03	0.06 ± 0.05	0.32 ± 0.16	0.04 ± 0.03	0.16 ± 0.06	0.14 ± 0.09
Oa	0.01 ± 0.01	0.03 ± 0.01	0.28 ± 0.20	0.06 ± 0.03	0.31 ± 0.14	0.29 ± 0.23
Oy	0.01 ± 0.01	0.03 ± 0.02	1.27 ± 0.74	0.21 ± 0.19	1.37 ± 0.52	8.01 ± 4.71
Incidence (%)						
AG2-1	48	11	50	71	69	62
Rc	69	44	69	41	73	40
Oa	60	96	90	90	96	98
Oy	67	93	98	88	94	98

Note: R. solani (AG2-1), *R. cerealis* (Rc), *O. acuformis* (Oa), *O. yallundae* (Oy). 2011/12 $n = 52$, 2012/13 $n = 50$.

TABLE 4 Pathogen DNA in stem bases (5 cm stem basal region) of English winter wheat plants collected at growth stage (GS) 21-31, GS 37-45, and GS 65-75 in 2011/12 and 2012/13

	GS 21–31		GS 37–45		GS 65–75	
Pathogen	**2011/12**	**2012/13**	**2011/12**	**2012/13**	**2011/12**	**2012/13**
Fungal DNA (pg/ng of total DNA) mean ± 95% CI						
AG2-1	0.0004 ± 0.001	0 0.00002 ± 0.00002	0.0001 ± 0.0001	0.00008 ± 0.00004	0.001 ± 0.0004	0.001 ± 0.001
Rc	1.83 ± 0.70	0.09 ± 0.07	2.31 ± 0.94	0.16 ± 0.10	1.46 ± 0.62	1.19 ± 0.92
Oa	0.23 ± 0.12	0.04 ± 0.02	1.85 ± 1.53	0.17 ± 0.15	9.70 ± 5.07	4.59 ± 2.91
Oy	0.87 ± 0.52	0.10 ± 0.06	7.64 ± 3.64	2.14 ± 1.76	45.69 ± 14.27	110.90 ± 48.00
Mn	7.17 ± 1.50	0.50 ± 0.27	0.37 ± 0.20	0.70 ± 0.22	1.88 ± 1.26	3.15 ± 1.15
Mm	1.30 ± 0.50	0.17 ± 0.09	1.02 ± 0.43	1.23 ± 1.27	3.56 ± 3.22	2.59 ± 1.24
Incidence (%)						
AG2-1	43	13	21	40	64	51
Rc	96	50	96	48	94	61
Oa	100	94	100	96	100	98
Oy	100	94	100	96	100	98
Mn	96	97	96	96	100	92
Mm	92	90	92	98	100	100

Note: R. solani (AG2-1), *R. cerealis* (Rc), *O. acuformis* (Oa), *O. yallundae* (Oy), *M. nivale* (Mn), *M. majus* (Mm). 2011/12 $n = 52$, 2012/13 $n = 50$.

Root rot was weakly but positively correlated with each meteorological variable in the spring (Table 6). The strongest correlation was with relative humidity (RH) ($r = .50$). Eyespot DI at GS 21–31 was negatively associated with rainfall and RH over the autumn/winter period. The stronger positive correlations ($r > .55$) were with each meteorological variable in the spring and lower temperature and rainfall in the summer. BFR was only correlated with variables in the autumn/winter period, with positive correlations with rainfall and humidity and negative associations with $Temp_{max}$ and $Temp_{min}$ (Table 6). Sharp eyespot at GS 21–31 had a weak but positive correlation with rainfall over the autumn/winter period, whilst in the summer period sharp eyespot had strong correlations with rainfall ($r = .60$), relative humidity ($r = .47$), and $Temp_{max}$ ($r = -.50$).

3.5 | PCA for soil, roots, stem bases and stems

Associations at GS 21–31 between species and diseases with meteorological variables in the autumn/winter period are shown as PCA biplots in Figure 1 for soil (a), roots (b), and stem bases (c). PC1 and PC2 explained 59% of the variation in the soil biplot, and vectors for

TABLE 5 Pathogen DNA in stems (15 cm above the 5 cm basal region) of English winter wheat plants collected at growth stage (GS) 37–45 and GS 65–75 in 2011/12 and 2012/13

	GS 37–45		GS 65–75	
Pathogen	**2011/12**	**2012/13**	**2011/12**	**2012/13**
Fungal DNA (pg/ng of total DNA) mean ± 95% CI				
Rc	1.67 ± 0.85	0.004 ± 0.005	4.90 ± 2.03	6.74 ± 5.60
Oa	0.14 ± 0.09	0.91 ± 1.76	4.45 ± 3.19	0.41 ± 0.57
Oy	0.33 ± 0.20	1.55 ± 2.52	3.44 ± 2.11	2.33 ± 2.08
Incidence (%)				
Rc	89	10	90	78
Oa	89	80	98	76
Oy	94	76	98	98

Note: R. cerealis (Rc), *O. acuformis* (Oa) and *O. yallundae* (Oy). 2011/12 $n = 52$, 2012/13 $n = 50$.

TABLE 6 Correlation coefficients (r) and probabilities (in parentheses) if $p \leq .05$ between disease index on roots and stems assessed at growth stage (GS) 21–31, GS 37–45, and GS 65–75 in 2011/12 to 2012/13 and temperature (maximum and minimum), total rainfall, and relative humidity in English winter wheat fields

	Root rot	Eyespot	Brown foot rot	Sharp eyespot
$Temp_{max}$ (°C)				
Autumn/winter	ns	.37 (<.001)	−.44 (<.001)	ns
Spring	.34 (<.001)	.59 (<.001)	ns	.31 (.002)
Summer	ns	−.49 (<.001)	ns	−.50 (<.001)
$Temp_{min}$ (°C)				
Autumn/winter	ns	.33 (<.001)	−.41 (<.001)	ns
Spring	.38 (<.001)	.60 (<.001)	ns	.40 (<.001)
Summer	ns	ns	ns	ns
Total rainfall (mm)				
Autumn/winter	ns	−.33 (<.001)	.53 (<.001)	.24 (.018)
Spring	.35 (<.001)	.59 (<.001)	ns	.29 (.003)
Summer	ns	.57 (<.001)	ns	.60 (<.001)
Relative humidity (%)				
Autumn/winter	−.25 (.011)	−.22 (.026)	.26 (.009)	.21 (.032)
Spring	.50 (<.001)	.59 (<.001)	ns	.26 (.009)
Summer	ns	.38 (<.001)	ns	.47 (<.001)

Note: Autumn/winter, presowing to GS 21–31; spring, GS 21–31 to GS 37–45; summer, GS 37–45 to GS 65–75. ns, not significant, $p > .05$.

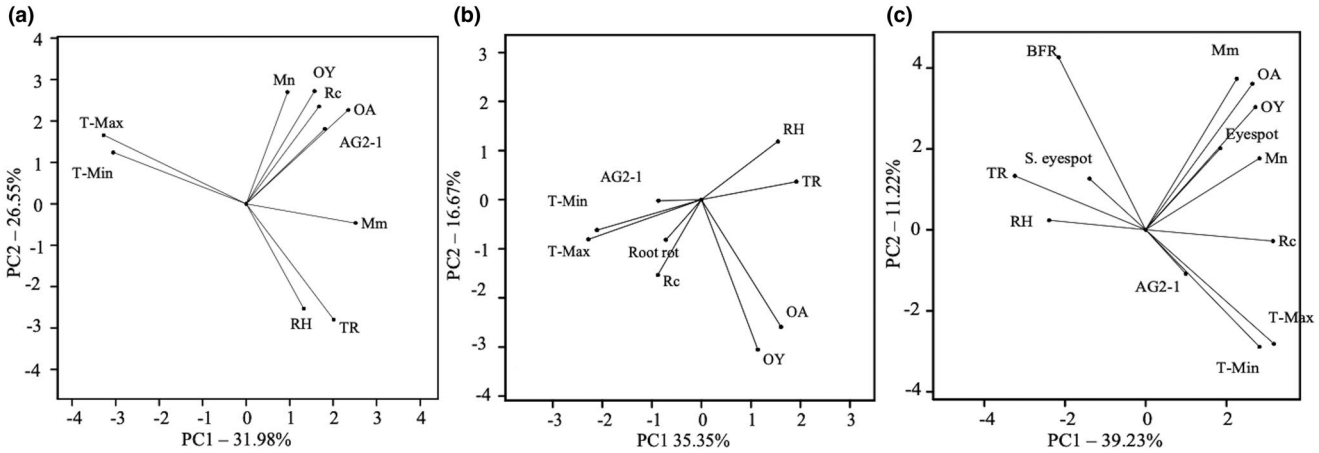

FIGURE 1 Associations between DNA of stem-base pathogens, their diseases, and meteorological variables in (a) soil, (b) roots, and (c) stem bases of winter wheat at growth stage (GS) 21–31 shown as biplots using principal component analysis. *Microdochium nivale* (Mn), *M. majus* (Mm), *Oculimacula acuformis* (OA), *O. yallundae* (OY), *Rhizoctonia cerealis* (Rc), *R. solani* AG 2-1 (AG 2-1), and disease index for eyespot, sharp eyespot (S. eyespot), and brown foot rot (BFR). Total rainfall (TR), relative humidity (RH), and mean daily maximum (T-max) and minimum (T-min) temperature

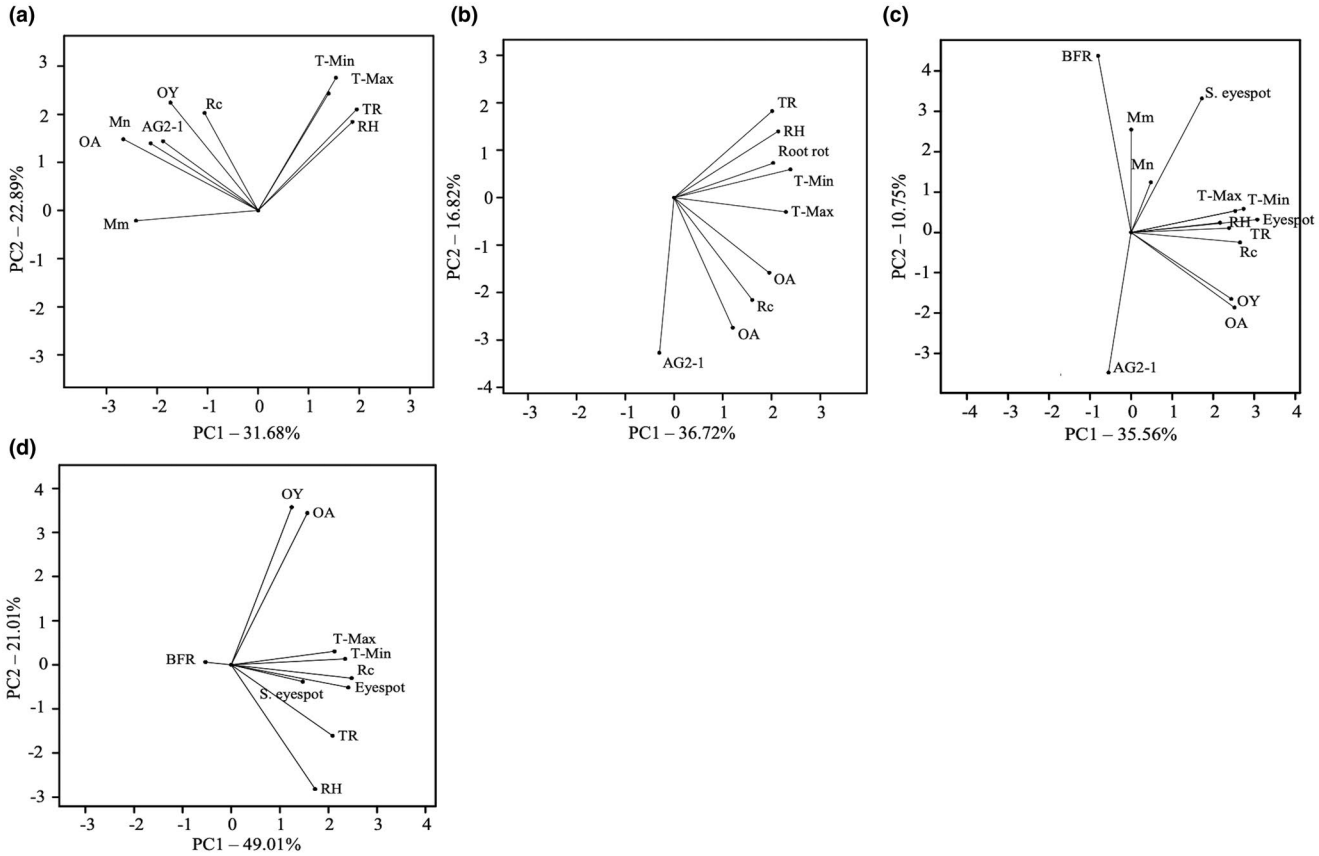

FIGURE 2 Associations between DNA of stem-base pathogens, their diseases, and meteorological variables in (a) soil, (b) roots, (c) stem bases, and (d) stems of winter wheat at growth satge (GS) 37–45 shown as biplots using principal component analysis. *Microdochium nivale* (Mn), *M. majus* (Mm), *Oculimacula acuformis* (OA), *O. yallundae* (OY), *Rhizoctonia cerealis* (Rc), *R. solani* AG 2-1 (AG 2-1), and disease index for eyespot, sharp eyespot (S. eyespot), and brown foot rot (BFR). Total rainfall (TR), relative humidity (RH), and mean daily maximum (T-max) and minimum (T-min) temperature

all species, except for *M. majus*, were clustered together along the *y* axis showing no association with any of the meteorological variables. The root biplot (Figure 1b) weakly grouped root rot and *R. cerealis* together. However, the relatively short vector of root rot indicated that this factor had a minor influence on the variation in this data set. *Oculimacula* spp. grouped together showing no association with the other pathogens or root rot. The stem bases biplot (Figure 1c) clustered *Oculimacula* spp. and *Microdochium* spp. with eyespot disease index. BFR showed independence from *Microdochium* spp. Sharp eyespot was clustered with rainfall and humidity but showed a negative association with its causal agent *R. cerealis*.

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

At GS 65–75, associations between species and meteorological variables are shown as PCA biplot in Figure 3 for soil (a), roots (b), stems bases (c), and stems (d). In the soil biplot (Figure 3a; PC1 and PC 2 = 56%) species, except *M. majus*, were again clustered in two groups. *O. yallundae*, *R. cerealis*, and AG2-1 grouped together, whilst *O. acuformis* and *M. nivale* were associated with $Temp_{min}$. In the roots biplot (Figure 3b; PC1 and PC2 = 53%), root rot was not associated with any of the pathogens. However, again the short vector for root rot suggested this factor had a minor effect on the variation in this data set. *Oculimacula* spp. and *R. cerealis* were again grouped together, suggesting an association between these species in roots. The biplot for stem bases (Figure 3c; PC1 and PC2 = 46%) showed eyespot closely aligned to *R. cerealis* rather than to *Oculimacula* spp., which grouped with *Microdochium* spp. and $Temp_{min}$. Sharp eyespot was grouped with total rainfall and relative humidity but was

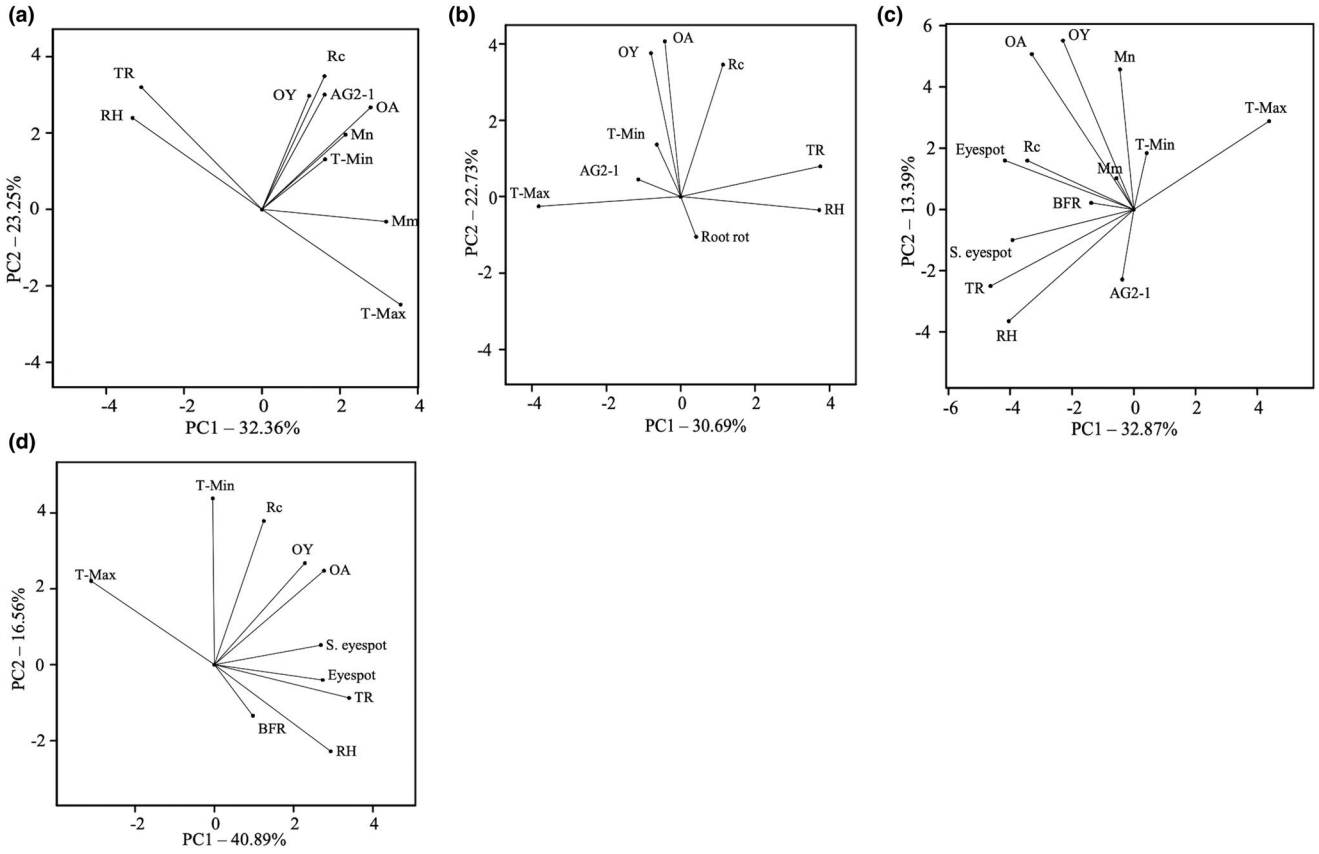

FIGURE 3 Associations between DNA of stem-base pathogens, their diseases, and meteorological variables in (a) soil, (b) roots, (c) stem bases, and (d) stems of winter wheat crops at growth stage (GS) 65–75 shown as biplots using principal component analysis. *Microdochium nivale* (Mn), *M. majus* (Mm), *Oculimacula acuformis* (OA), *O. yallundae* (OY), *Rhizoctonia cerealis* (Rc), *R. solani* AG 2-1 (AG 2-1), and disease index for eyespot, sharp eyespot (S. eyespot), and brown foot rot (BFR). Total rainfall (TR), relative humidity (RH), and mean daily maximum (T-max) and minimum (T-min) temperature

only loosely associated with its causal agent *R. cerealis* aligning with *Oculimacula* spp. In the stems biplot (Figure 3d; PC1 and PC2 = 57%), sharp eyespot and eyespot were clustered along the *x* axis along with rainfall, but only weakly associated with their causal agents.

4 | DISCUSSION

This is the first study to quantify the dynamics of a range of fungal species and diseases in soil, root, and stem fractions of English wheat crops and as such provides novel information on the incidence of economically important wheat pathogens, and the risk of their associated diseases. The novel finding of this study is that the predominant *Rhizoctonia* spp. in soil of English wheat crops was *R. solani* AG 2-1, occurring on average in 63% of fields. This is in contrast to a previous soil survey by Goll et al. (2014) using a soil baiting method, which isolated AG 2-1 in just 13% of soil samples ($n = 60$) from arable fields in the UK. Here, we used species-specific qPCR assays to acquire more accurate quantification of targeted pathogens than soil baiting, although the soil baiting method provides useful information on the viability of pathogens within the soil profiles. This difference in methodology could explain the higher percentage of fields containing AG 2-1 in our study. The widespread distribution of AG2-1 globally has been demonstrated by previous studies in wheat-growing regions of the USA (Schroeder et al., 2011) and in potato crops in south-eastern Australia (Sparrow et al., 2015). Less is known of the aggressiveness of UK AG 2-1 isolates to wheat in England, but the decline of the pathogen in soils over the seasons suggests the exhaustion or the lack of suitable substrate to sustain its continued accumulation. We found low DNA concentrations of AG2-1 in wheat roots/stems, and together with the lack of significant associations with any assessed disease, this suggests that winter wheat is unlikely to be a major host for this pathogen. Indeed, previous studies by Sturrock et al. (2015) have shown that AG2-1 is less pathogenic to wheat than to oil-seed rape (OSR), thus it is more likely that wheat serves as an alternative host for survival of AG2-1 until the more susceptible OSR crop is planted in the rotation. AG2-1 is highly pathogenic to OSR (Babiker et al., 2013) with much more significant implications for yield loss.

O. acuformis was the most frequently detected species from the SBD complex, occurring on average throughout both seasons in 73% of field soil samples, followed by *O. yallundae* (68%), *R. solani* AG 2-1 (63%), and *R. cerealis* (54%), confirming that the soil medium is used by these pathogens both for survival and as an inoculum source for the

initiation of their diseases. Overall, *M. nivale* and *M. majus* were the least detected species in terms of incidence or biomass. However, the incidence of *M. nivale* from presowing over the autumn/winter period increased by >100% in both seasons. This suggests that *Microdochium* spp. may have been introduced into fields on infected seed, which is generally accepted as the most important source of inoculum for these pathogens (Parry et al., 1995). PCA showed that all pathogens except *M. majus* were generally positively grouped together in the soil, suggesting coexistence. The population densities in recovered roots showed that the most commonly occurring species was *O. yallundae* (90%) rather than *O. acuformis* (88%). These were followed by *R. cerealis* (56%) and *R. solani* AG 2-1 (52%). The majority of wheat crops in England exhibited root rot symptoms of low to moderate severity, with a peak at GS 21–31, followed by a decline as plants matured. Although *R. cerealis* DNA was frequently detected (56%) on roots, previous research has shown that this species does not cause any disease on wheat roots (Sweetingham et al., 1986). Therefore, it is not known what role, if any, the occurrence of *R. cerealis* or *Oculimacula* spp. on roots has on the epidemiology of sharp and true eyespot, respectively, or what effect root infection by these pathogens would have on the plant and ultimately yield. Because there were no relationships between root rot and the pathogens in this study in the PCA, root rot symptoms here may have been caused by other abiotic or biotic factors, including other species that may have not been identified in our studies (Raaijmakers et al., 2009). The positive associations between *Oculimacula* spp. and *R. cerealis* grouping in the root PCA at GS 37–45 and GS 65–75 suggest a common survival strategy by below-ground colonization of root residues until the next susceptible host is planted.

M. nivale was the predominant species in stem bases at GS 21–31, particularly in 2011/12, suggesting that this species was able to colonize the stem base over the autumn/winter period to a greater extent than the other pathogens. As *Microdochium* spp. were the least frequently detected pathogens in soil, seedborne inoculum may have contributed to early systemic stem colonization. BFR remained the predominant SBD throughout the crop growing seasons in both years, reaching 100% incidence by GS 37–45. Hardwick et al. (2001) also showed in studies over more than 10 years that BFR was the most commonly occurring stem disease in more than 89% of UK wheat crops. The only strong correlations for BFR were with temperature and rainfall over the autumn/winter period, suggesting BFR was more severe in cooler and wetter areas. This was shown at GS 21–31 when BFR index was 68% higher in 2012/13, coinciding with rainfall over the autumn/winter, which was 121% higher than in 2011/12. BFR remained relatively constant at GS 37–45 and GS 65–75 between seasons, suggesting variation in environmental conditions had little effect on the development of disease as the crop matured.

Bateman (1993) first reported that *Oculimacula* spp. were coisolated with *M. nivale* more frequently than would be expected by chance, which agrees with our results from the PCA of the stem base pathogen complex at GS 21–31 and GS 65–75 showing a strong association. Furthermore, the most frequently co-occurring species on the stem bases were *Oculimacula* and *Microdochium* spp. in ≥90% of crops in both seasons, suggesting that these species generally coexist. *O. acuformis* and *O. yallundae* also clearly grouped together at each growth stage, indicating a strong positive association. Changes in the populations of *Oculimacula* spp. due to differences in fungicide sensitivity have been documented (Turner et al., 2002). Our studies indicate that the most recent shift is in favour of *O. yallundae*, as this species was found in higher DNA concentration in stems and roots throughout the growing seasons. This was most notable at GS 65–75 in 2012/13 when DNA concentrations of *O. yallundae* were 24-fold higher than of *O. acuformis*.

The seasonal incidence and severity of eyespot and sharp eyespot across sites were higher in 2011/12 compared to 2012/13 and both correlated at GS 37–45 with higher temperature and rainfall in the spring. As *Oculimacula* spp. grouped separate from *R. cerealis* at this stage, it is likely that other factors not included in our analysis may have affected their interactions. Rainfall (McCartney & Fitt, 1998) and temperature (Bock et al., 2009) are the two environmental factors positively influencing eyespot development and establishment on stems. However, as both sharp eyespot and eyespot failed to establish on stems in 2012/13 at GS 37–45 and GS 65–75 when total rainfall in the summer period was only 34 mm compared to 180 mm in 2011/12, rainfall appears by far the more important factor influencing these two diseases. This agrees with our PCA at GS 65–75 showing closer associations between eyespot and sharp eyespot with total rainfall rather than any other environmental variable. In contrast to *Microdochium* and *Oculimacula* spp., which seemed to coexist in the lower 5 cm basal region, the preferred niche of *R. cerealis* at GS 65–75 was confirmed to be 15 cm above this region, shown by the higher DNA concentrations of the pathogen in this fraction in both seasons.

Our results also show that visual disease assessments of stems do not agree strongly with individual pathogen DNA in planta. For example, at GS 37–45 BFR and sharp eyespot were grouped with *Microdochium* spp. whilst eyespot was associated with *R. cerealis*. Mixed infections in the SBD complex are known to be difficult to distinguish at early crop growth stages (Turner et al., 1999, 2001). However, in our studies eyespot and sharp eyespot were more often misdiagnosed with their causal organisms than BFR. It is likely that this is because in time eyespot is an intermediary disease that occurs after BFR and prior to sharp eyespot. Thus, eyespot symptoms correlated with both *Microdochium* and *Oculimacula* spp. on wheat stems up to GS 21–31 and then with *R. cerealis* beyond stem extension. This is further supported by the positive associations on the wheat host between *M. nivale* and *O. acuformis* whilst *R. cerealis* was not associated with *Microdochium* spp., suggesting the latter two species are separated in time and space or in competition. Our results showing visual misdiagnosis of sharp eyespot and eyespot have important implications for disease control because fungicides differ in their efficacy against their individual pathogens, thus making a strong case for the integration of molecular-based solutions to aid treatment decisions.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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