



# Identifying potential novel resistance to the foliar disease ‘Scald’ (*Rhynchosporium commune*) in a population of Scottish Bere barley landrace (*Hordeum vulgare* L.)

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Received: 24 November 2020 / Accepted: 26 April 2021 / Published online: 27 May 2021  
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## Abstract

Barley ‘Scald’ is an economically damaging fungal disease that is a global problem, causing significant yield and economical losses in the UK barley feed and malting industries. Presently, a limited number of *Rhynchosporium* resistance genes exist, but selective pressures on the fungi cause the demand for new sources of resistance. Landraces, such as the Scottish Bere barley, hold potential sources of resistance that can be utilised, with farmers providing anecdotal evidence of resistance in field populations of Bere. This study analysed 131 heritage cultivars and landrace lines, including 37 Bere lines, to screen for resistance using both detached leaf assays (DLAs) and field experiments. Results showed that Bere lines produced smaller, but more necrotic, lesions for the majority of isolates in the DLAs, as well as smaller scores when visually assessed in field conditions. Whilst the infection patterns of the lines differed between isolates and experimental conditions, three Bere lines were identified as consistently showing reduced levels of infection (45 A 23, 58 A 36 Eday, and 8-125). Using genome-wide association analysis, we were able to identify a number of genomic regions associated with reduced infection symptoms, four in regions associated with known resistance genes, but another four associated with new regions that contain promising candidate genes. Further analysis of these new regions and candidate genes should be undertaken to identify targets for future disease-resistance breeding.

**Keywords** Barley landraces · *Hordeum vulgare* · Bere barley · Genetic diversity · *Rhynchosporium commune* · Barley leaf scald

## Introduction

The *Rhynchosporium* genus comprises of haploid hemibiotrophic fungi that infect grasses such as rye (*Secale cereale*), triticale (*×Triticosecale* spp.), and barley (*Hordeum vulgare*). Originally, the pathogens of these three crops were classified as pathotypes of *R. secalis*. A study by Zaffarano et al. (2011) identified that the pathotype infecting barley

was a separate species, and it was subsequently labelled as *Rhynchosporium commune*. This host adaptation is mediated through effector proteins that stall the development of the fungi *in planta* in order to extend the biotrophic phase (Clark et al. 2008; Penselin et al. 2016). The disease, known as ‘barley leaf blotch’, ‘scald’ or ‘rhynchosporium’, is found in all areas of the world where barley is grown, but is especially prevalent in areas with cooler, wetter climates. Yield losses have been reported ranging from 10 to 45%, and economic yield is reduced due to inferior quality grain for products such as malting barley (Avrova and Knogge 2012). In the UK, this equates to an estimated £7.2 million worth of losses in barley after fungicide treatments (Paveley et al. 2016).

Whilst the fungus can be seed-borne, the majority of infection comes from the spread of the fungus originating on crop debris from the previous season, or from volunteer plants that host the fungus over winter. This spread is usually via rain splash which causes dispersal of spores to new hosts; air-borne transfer of spores may also transmit

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the pathogen but is thought to be much more limited (Fontaine et al. 2010). Seed-borne infection is thought to remain symptomless *in planta* for several months (Clark et al. 2008; Fontaine et al. 2010; Avrova and Knogge 2012). Infection through rain splash dispersal can also remain symptomless as the biotic phase does not produce the characteristic symptoms of scald shown in other phases (Zhan et al. 2008). Current strategies to reduce the number of rhynchosporium outbreaks include: agronomic practices such as crop rotations, fungicide use, and genetic resistance through breeding. Cultivar mixtures have also been shown to be effective, but are not currently a practiced breeding strategy (Newton et al. 1997; Wolfe et al. 1997). However, due to rapid adaptation of the pathogen populations, individual treatments are often inadequate by themselves (Zhan et al. 2008). This rapid adaptation of pathotypes—in response to changing environments, resistant cultivars, and fungicide treatments, is thought to be caused, in part, by the sexual recombination of the pathogens that occurs in all field populations (McDonald 2015). This causes selective pressures that account for approximately three quarters of the global *R. commune* species diversity found in the field (McDonald 2015) and an increasing race complexity with time (Zhan et al. 2012). Additionally, the prohibition of fungicides for regulatory reasons highlights the risk in the reliance on chemical treatment as the sole form of control. An example of this is the EU's imminent ban of the most commonly used fungicide in the USA and UK—chlorothalonil (Hillocks 2012; European Commission 2019). Together these show a need for the development of more durable genetic resistance to *R. commune*.

Currently, a limited number of resistance genes in barley against *R. commune* have been identified, with *Rrs18*, found on chromosome 6HS by Hofmann (2014) and confirmed by Coulter et al. (2018) being the most recently reported. A number of these *Rrs* genes originate from landraces (Bjørnstad et al. 2004; Hanemann et al. 2010; Hofmann et al. 2013). It is suggested that Scandinavian and other northern European landraces might be a prime source for resistance to rhynchosporium as it is speculated that *R. commune* originated in this area providing diverse *R. commune* populations and the longest period of landrace selection for rhynchosporium resistance (McDonald 2015), suggesting the Scottish barley landrace population 'Bere' as a suitable candidate.

Bere barley is a six-row spring barley landrace that has had populations grown on predominately marginal land for at least the last half millennia, and currently is grown in the highlands and islands of Scotland. Historically, it was Scotland's main barley crop and was used for all barley uses of that time including food, feed, and malting, with the straw being used for animal bedding and thatching (Martin et al. 2009).

Within the literature, Bere barley is loosely referred to as a variety but is often shown to have different, contrasting phenotypes (SASA 2015; Wallace et al. 2019). Maintained on farm, Bere is a population potentially comprising much variation. This diversity is due to the isolated nature of the islands where Bere is still grown. A study undertaken by Southworth (2007) using 29 microsatellite markers shows that there is significant genetic variance between the 3 Scottish island groups of the Shetland, Orkney, and Western Isles. It was suggested that this clustering is due to the lack of historical seed trade between the island groups, but also suggested that it could be due to adaptation to the differing environments of the islands. This diversity was compared with the diversity of 134 barley cultivars on the UK national list, over 5 loci, and showed that the Bere lines have similar levels of diversity as found in the pool of elite cultivars. Whilst this diversity between island groups was high, over two-thirds of the total diversity was found within the island group clusters. The Western Isles displayed the greatest genetic diversity, potentially due to similar geological constraints as between the island clusters.

It has been observed that Bere barley has an increased susceptibility to powdery mildew (*Blumeria graminis* f.sp. *hordei*), and possibly other foliar diseases (Wright et al. 2002) such as barley leaf stripe (*Pyrenophora graminea*), when compared to other Scottish barley seed (Cockerell 2002). However, in an interview in 2003 with farmers who grew Bere barley in Orkney, the susceptibility to powdery mildew was not noted by most as a concern (Martin et al. 2009). This difference in reported and observed susceptibility could be due to differences within the Bere population. Later interviews with farmers by Mahon et al. (2016) suggest that Bere are more resistant to other foliar diseases, in particular rhynchosporium. This supports the suggestion that Bere barley is a prime source for resistance to rhynchosporium.

This study investigates inherent resistance to rhynchosporium in Bere lines, as suggested by anecdotal evidence (Mahon et al. 2016). Identifying individual lines that have high resistance along with associated genomic regions and candidate genes in these regions.

## Materials and methods

### Barley material preparation

A total of 131 lines (Supplementary Table 1) from The James Hutton Institute (Dundee, UK) Heritage Spring Barley Landrace Collection (JHI-SBLC) were selected, with seeds predominantly originating from the JIC-GRU (John Innes Centre Germplasm Resources Unit) or SASA (Scottish Agricultural Science Agency) collections. This contained

landraces, including 37 Bere lines, and other heritage cultivars. The lines were multiplied in glasshouse conditions as outlined in Cope et al. (2020).

### Maintenance and isolation of *R. commune* spores

Isolates of *R. commune* were previously obtained from regions within the UK. Isolates L77, L73A, and L2A were acquired through the RhynLINK project in 2007 grown in Dundee from farm saved seed obtained from Scottish Agronomy, or DuPont for the latter (Fitt et al. 2012). An additional isolate, 13-13, was acquired from the UK Cereal Pathogen Virulence Survey (ahdb.org.uk/UKCPVS). Samples of these isolates were selected from the stored collection at the James Hutton Institute on silicon beads stored at  $-20\text{ }^{\circ}\text{C}$ . Fungal isolates were grown in 10 cm Petri dishes on a vegetable-based medium, CZCMV8 (Newton and Caten 1988). The samples were then sealed and incubated in the dark at  $17\text{ }^{\circ}\text{C}$  for 2–3 weeks.

Isolates were suspended in distilled water, then filtered through glass wool in a funnel and centrifuged three times at 3000 g for four minutes to remove the excess debris and soluble germination self-inhibitors (Ayres and Owen 1970). The inocula were then each diluted to achieve a concentration of  $1 \times 10^5$  as measured using a haemocytometer.

### Landrace screen

Three seeds each from 122 lines (Supplementary Table 1, reduced selection due to seed availability), along with ten elite cultivars (Belgravia, Concerto, KWS Irina, Odyssey, Optic, Propino, RGT Planet, Scholar, Waggon, and Westminster) were randomised, in a complete randomised design, and grown in  $6 \times 10$  trays for approximately 20 days in universal compost—made as outlined in Cope et al. (2020). The glasshouse was maintained at day/night temperatures of  $18/14\text{ }^{\circ}\text{C}$  and 16 h daylight, with supplementary lighting (at  $200\text{ }\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$ ) provided when light intensity was less than  $200\text{ W m}^{-2}$  and shading when above  $450\text{ W m}^{-2}$ .

Four leaf sections were taken from each line/cultivar and randomised across  $79 \times 47 \times 21$  mm inoculation boxes (Stewart Plastics Ltd., Surrey, UK) containing 2% water agar and 0.3% (w/v) benzimidazole (Sigma, UK). The leaf segments had the waxy surface layer disrupted using a brush and were then inoculated by applying  $10\text{ }\mu\text{l}$  of the appropriate spore suspension (one isolate replication per line/cultivar) and incubated in a controlled environment cabinet (Leec, model LT1201) at  $17\text{ }^{\circ}\text{C}$ , with 24-h light ( $200\text{ lx s}^{-1}$ ), for 2–3 weeks (Newton et al. 2001). This was repeated four times to get a total of 5 replications for each line with each cultivar. Photographs were taken after the visual symptoms appeared and before leaf senescence of any line/cultivar; photographs were taken of all samples within each rep at the

same time. The size of the lesion was then measured using the Java-based image processing program ‘ImageJ’ (Schneider et al. 2012), and the intensity of the disease (noted by area of the lesion necrotised) was scored on a scale of 0–4—where 0 is no necrosis and 4 is necrosis in  $>90\%$  of the lesion (Supplementary Fig. 1).

### Field trial screen

Two field trials were sown at the irrigated JHI rhynchosporium nursery over two years with 50 lines in 2016 (12th April; reduced lines due to seed availability) and 130 lines plus 10 elite cultivars (as above) in 2017 (20th April; Supplementary Table 1, reduced selection due to seed availability). The lines/cultivars were randomised and sown into one of four linear arrays in the  $1 \times 1.55$  m plots at a rate of 20 seeds per array, with two replicas per line/cultivar for each year. A previously planted rhynchosporium susceptible winter barley mix was used as a guard and to propagate the pathogen. Sprinkler irrigation was used regularly to encourage splash dispersal of spores and free water for infection. Samples were scored from 1 to 9, from no symptoms to 100% necrotic leaves as detailed in AHDB (2015), at multiple time points between 58 and 91 days after sowing—with 11 days between scoring.

### Genotypic data

Germination, DNA isolation, genotyping, and analysis were carried out previously to obtain the genotypic data (Cope et al. 2020). The genotypic data was processed by removing the markers that had a low call rate ( $<80\%$ ) or low minor allele frequency ( $<10\%$ ), along with the genotypic lines that had a low rate of marker return ( $<80\%$ ) or large heterozygosity (False Discovery Rate  $<10\%$ ). The statistical program R (R Core Team 2013), with the GenABEL package (Aulchenko et al. 2007), was used to perform GWAS using a mixed linear model (MLM) approach controlling for population structure and relatedness, as outlined in Yu et al. (2006) and Zhang et al. (2010), with the lesion size and severity data for the average isolate and individual isolate data. Quality controls for the GWAS were performed using quantile–quantile (QQ) plots. The region of the QTL was identified by a LOD drop of greater than two, or the range between the first and last marker in the peak  $\pm 0.5\text{ Mbp}$ , taking whichever was the greater range. This region was then searched using the Barlex database (with the Morex v2 Gene Models) for genes contained within (Colmsee et al. 2015).

### Statistical analysis

Landrace screen data was analysed using an unbalanced ANOVA with Genstat 18th edition (VSN International, UK),

separately for the variates lesion severity and area of infection. The treatment for each was the line/cultivar and isolate, with the blocking factor being position within box number within experiment.

Field trial score data was transformed, by  $\log_{10}(+1)$ , then used to calculate a single disease score using the relative area under the disease progress curve (RAUDPC) values for each line based on the score over different time points (Campbell and Madden 1990). Each year's data was analysed separately using an unbalanced ANOVA for this variate. The treatment was the line, with blocking factors of column within row within cassette (within bed for the 2017 trial, and within year when combined). This was repeated to analyse the data when combined into three sub-categories: Bere, elite cultivars, other landraces/heritage cultivars—the latter being made up from the non-Bere lines of the JHI collection. Only the 2017 field trial data was selected for use in a GWAS.

Markers were removed, based on the criteria above and from the set of 37242 used, for having low minor allele frequency and a low call rate (Supplementary Table 2), and barley lines we excluded due to high levels of heterozygosity and being identical by state (Supplementary Table 3). These removals were different between isolates, and from the field trial data, due to the removal on non-viable leaves. QQ plots were used to determine which model, mixed linear model or EIGENSTRAT, was the closest fit for each variable.

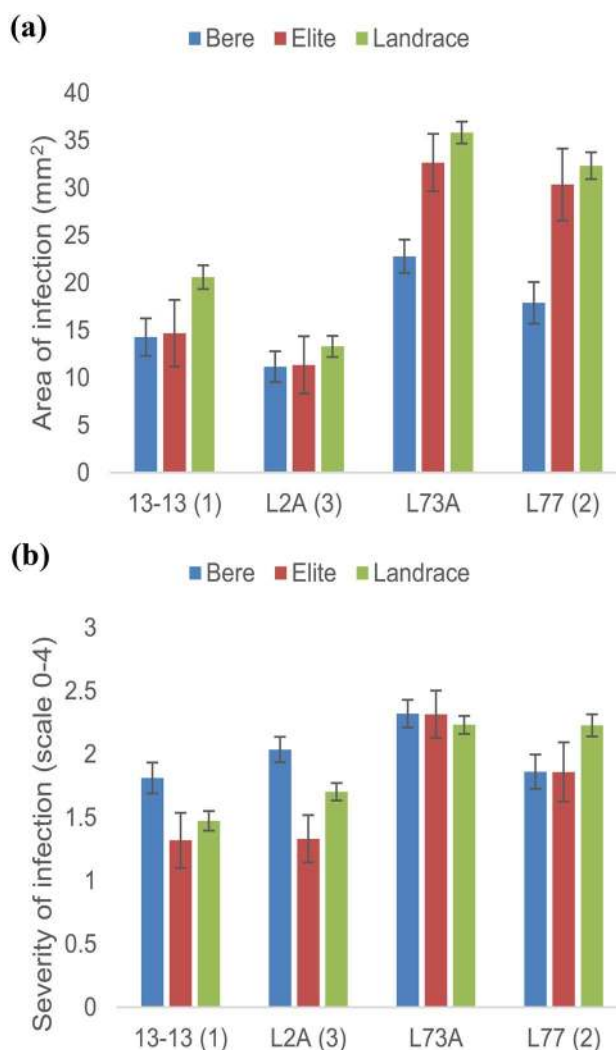
## Results

### Controlled landrace screen

#### Lesion analysis of barley sub-category

Significant differences were seen in lesion area between sub-categories ( $p < 0.001$ ), showing Bere lines exhibiting smaller lesions on average (Fig. 1a). Differences between isolates ( $p < 0.001$ ) were also seen with L2A and 13-13 producing smaller lesions at 13–19 mm<sup>2</sup> compared to 30–33 mm<sup>2</sup> in L73A and L77. Additionally, differential interaction of the variates ( $p = 0.043$ ) was observed with L73A and L77 causing smaller lesions in the Bere lines only, 13-13 in the Bere and Elites, and L2A showing no differences between sub-categories.

Significant differences in lesion severity were not observed between sub-categories ( $p = 0.066$ ), but were in the variate interaction ( $p = 0.048$ ) that showed that the Bere lines exhibited greater severity with isolates L2A and 13-13 (Fig. 1b). Differences were also seen between isolates ( $p < 0.001$ ) with L73A and L77 showing more severe lesions with minimal differences between the sub-categories.

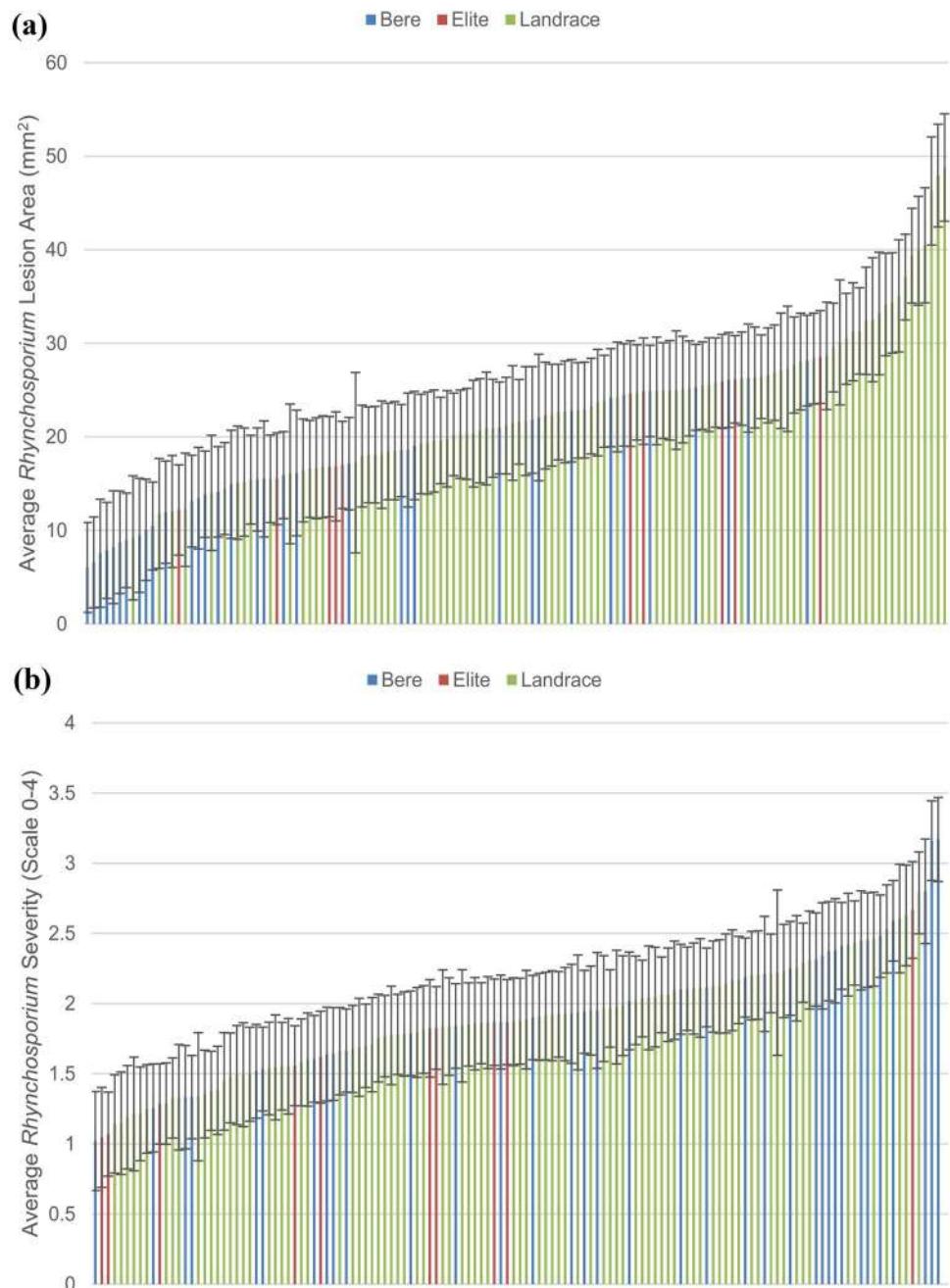


**Fig. 1** Comparison of the *R. commune* infection for 132 lines/cultivars of barley divided into three, unequal, sub-divisions (35, 87, and 10 for the Bere, other landraces, and elites, respectively) with four different isolates, looking at **a** area of infection, and **b** lesion severity of infection. Error bars represent the standard errors

#### Lesion analysis of individual lines

Individual analysis also shows significant differences between the lines/cultivars, isolates, and interaction of these treatments ( $p < 0.001$ ), for both lesion area and lesion severity. The lines in each sub-category show large variation in lesion area, but Bere lines were amongst those with the smaller lesions, with nine of the ten smallest lesions being on Bere lines (Fig. 2a), but with two elite cultivars within the SE of the smallest lesion. The lesion severity trait also showed wide variation within the sub-categories, showing the majority of the Bere lines with large lesion severity, with the highest three being Beres, but also with the lowest being a Bere line (Fig. 2b). The elite cultivars showed mostly small severity, except for Concerto.

**Fig. 2** Comparison of rhynchosporium symptoms for 132 lines/cultivars of barley, averaging the four different isolates, looking at **a** area of infection, and **b** lesion severity of infection. Error bars represent the standard errors

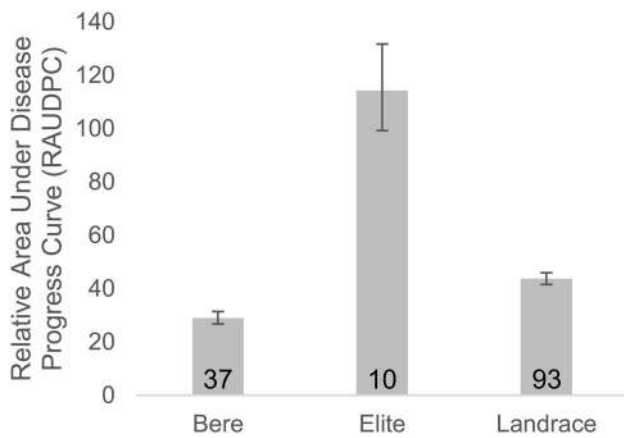


Comparison between lesion size and severity, for combined and individual isolate data, show no significant correlation. Similarly, comparisons between isolates for lesion area and severity showed no significant correlation. From this data however, line Bere 55 A 33 could be seen to exhibit small lesion sizes when infected with all isolates, and lines Bere 45 A 23, Bere 58 A 36 Eday, and Bere 8-125 produced small or no lesions when infected with all isolates, except isolate 13-13. Bere 55 A 33 also exhibited amongst the greatest severity but, similar to the other three lines identified above, had the smallest lesion size. Lines Spratt Archer 37/6/3-205 and Irish Goldthorpe-222 had exhibited

the largest lesion sizes with all isolates when infected; with Kenia-M08, Binder-M08, and Scotch Common-M08 when infected with all isolates except L2A.

### Field trials

Transformed data from the 2016 field trial showed no significant difference between the RAUDPC of individual lines ( $p=0.103$ ), but significance when the data was collated by sub-category ( $p=0.023$ ), indicating that there was a difference in rhynchosporium resistance (Supplementary Fig. 2). Similarly, significant differences were shown in the 2017



**Fig. 3** Comparison of rhynchosporium recorded as the relative area under disease progress curve (RAUDPC) during a field trial in 2017 for 140 lines/cultivars of barley divided into three, unequal, sub-divisions (37, 93, and 10 for the Bere, other landraces, and elites, respectively). Error bars represent the standard errors

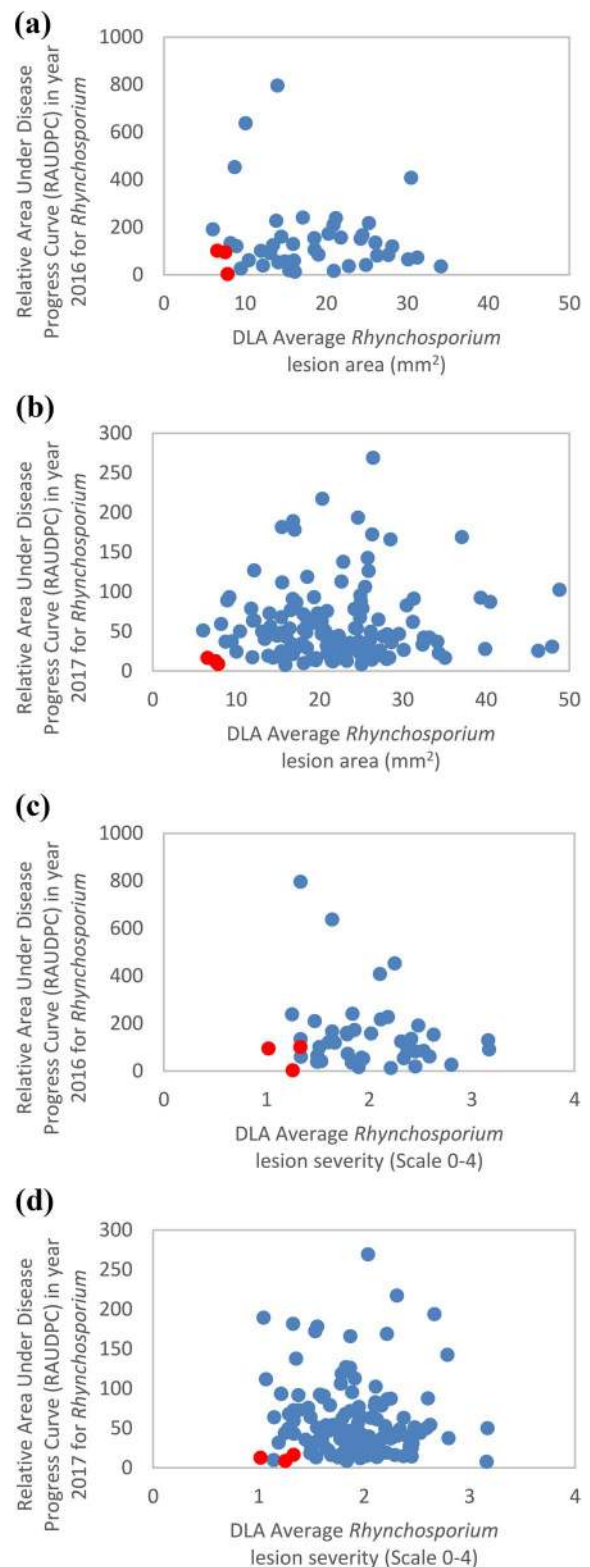
field trial data, showing differences between individual lines/cultivars ( $p < 0.001$ ) and sub-categories ( $p < 0.001$ ). Comparison of sub-categories shows large levels of infection in the elite cultivars, with approximately four times the RAUDPC of the Bere lines (Fig. 3 and Supplementary Fig. 3).

A comparison of the lines over the two field trials (Supplementary Fig. 4) shows no correlation between scoring data from the two years (correlation coefficient  $> 0.26$ ). Comparison of the field trial data from both years with the detached leaf assay average lesion area and lesion severity (Fig. 4) shows no significant correlations between the datasets. However, from these correlations three Bere lines were noted as having consistently low levels of infection over both years, these being Bere 45 A 23, Bere 58 A 36 Eday, and Bere 8-125.

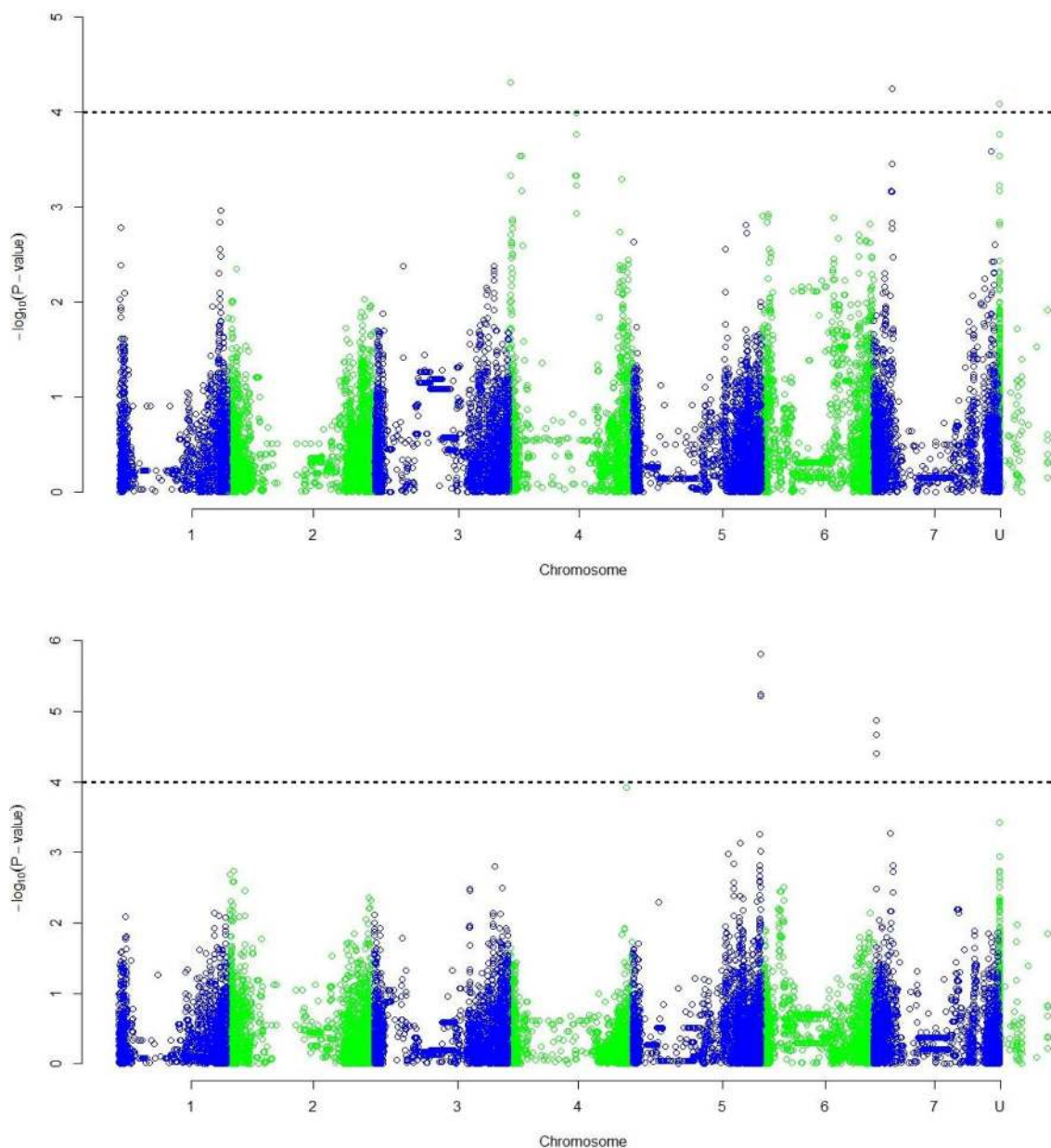
### Genome-wide association studies (GWAS)

Associations that were above the threshold of  $-\log_{10}(p \text{ value}) \geq 4$  were seen with isolate L2A and L77 for both lesion area and lesion severity. Isolate L2A (Fig. 5) showed significant associations on 4HS in the area data, 7HS in both the area and severity data, and the strongest statistical association on 5HL for lesion severity. Significant associations with isolate L77 (Supplementary Fig. 5) are seen on 2HL and 4HL in the lesion area data, as well as on 5HL in the lesion severity data.

Within chromosome 5HL, the regions identified by significant markers from isolate L2A and L77 lesion severity do not overlap. The most significant region with seven significant markers was from the L2A data at 667.34–667.59 Mb had 50 genes (Supplementary Table 4) of which one is



**Fig. 4** Correlation between the DLA average lesion area (a, b) or lesion severity (c, d) with the field trial RAUDPC from the years 2016 (a, c) or 2017 (b, d) with the respective 50 or 140 lines/cultivars used. The three lines—Bere 45 A 23, Bere 58 A 36 Eday, and Bere 8-125—that were shown to have consistently low infection are highlighted in red



**Fig. 5** A Manhattan plot of a Genome-Wide Association Study undertaken using an EIGENSTRAT approach on the lesion area (**a**), and lesion severity (**b**) when infected with *R. commune* isolate L2A; data generated using an ANOVA. Depressions in marker significance

observed in the centre of each chromosome are due to reduced marker density around the centromere of the physical map. The dotted line denotes a significance threshold of a  $p$  value  $< 0.0001$

highlighted, with the putative function of a receptor kinase with a domain homologous to Ginkbilobin-2 (Gnk2)—an antifungal protein found in *Ginkgo biloba* (Miyakawa et al. 2014) (Table 1). The regions associated with L77 lesion severity were located at 568.39 and 570.01, each with two significant markers. These regions contained 43 and 75 genes, respectively (Supplementary Table 4). Genes of note because of their putative function include (Table 1): four zinc-finger domains (including three RING-type), a MATE efflux family protein, a cathepsin B-like cysteine proteinase,

two pathogenesis-related thaumatin superfamily proteins, and two defensin/defensin-like proteins. Of these, zinc finger domains are relatively common comprising over 1.8% of all annotated genes with putative known functions in barley, at 902 of the 73586 genes annotated in BARLEX (the barley genome explorer), plus an additional 425 with a Zinc finger RING-type domain (Colmsee et al. 2015). Putative proteins annotated as MATE efflux family proteins, account for 94 proteins, or 0.13% of annotated genes. The defensins, thaumatin, cathepsins, and Gnk2 domain containing proteins

**Table 1** The name of the candidate genes, based on putative function, identified in relation to rhynchosporium lesions in detached leaf assays and field trial, with the chromosome and position on the physical map listed

Gene name	Chr	Position	Annotation
HORVU2Hr1G102740.3	chr2H	697.86	WRKY DNA-binding protein 35
HORVU2Hr1G102790.1	chr2H	698.16	Leucine-rich repeat receptor-like protein kinase family protein
HORVU2Hr1G107970.1	chr2H	713.07	Disease-resistance protein (CC-NBS-LRR class) family
HORVU2Hr1G108000.2	chr2H	713.11	Disease-resistance family protein / LRR family protein
HORVU2Hr1G108070.9	chr2H	713.27	Glycosyltransferase family 61 protein
HORVU2Hr1G108130.1	chr2H	713.44	Cysteine-rich receptor-like protein kinase 29 (with Gnk2 domain)
HORVU3Hr1G017430.1	chr3H	44.42	Cysteine-rich receptor-like protein kinase 2 (with Gnk2 domain)
HORVU5Hr1G082790.6	chr5H	568.39	RING/U-box superfamily protein
HORVU5Hr1G082810.1	chr5H	568.48	Cathepsin B-like cysteine proteinase
HORVU5Hr1G082830.1	chr5H	568.53	RING/U-box superfamily protein
HORVU5Hr1G082840.1	chr5H	568.56	RING/U-box superfamily protein
HORVU5Hr1G082890.5	chr5H	568.58	MATE efflux family protein
HORVU5Hr1G083240.3	chr5H	569.56	Pathogenesis-related thaumatin superfamily protein
HORVU5Hr1G083260.3	chr5H	569.57	Pathogenesis-related thaumatin superfamily protein
HORVU5Hr1G083320.1	chr5H	569.91	Defensin
HORVU5Hr1G083330.1	chr5H	569.91	Defensin-like protein 1
HORVU5Hr1G083760.1	chr5H	570.52	Zinc finger BED domain-containing protein RICESLEEPER 1
HORVU5Hr1G124810.4	chr5H	667.43	Receptor kinase 1 (with Gnk2 domain)

conversely represent < 0.1% of genes combined, with 17, 45, 64, and 68 genes found across the genome, respectively.

The peak at chromosome 7HS from the L2A lesion severity data had five significant markers at 16.67–16.92 Mb; this did not align with the indicated region in the 13–13 area data at 10.15–10.94 Mb. These could possibly correlate to *Rrs12* (Abbott et al. 1992) and *Rrs2* (Hanemann 2009), respectively. Likewise, the markers identifying a significant region on 4HS in the L2A area data possibly correlate with the location of *Rrs16* (Pickering et al. 2006), and the large region on chromosome 4HL between 572.61 and 596.53 Mb, could co-locate with *Rrs3* (Bjørnstad et al. 2002).

The significant marker on 2HL in the isolate L77 area data at 697.86 Mb possibly correlates to a QTL found by Backes et al. (1995) that associated with rhynchosporium resistance and that greatly affected barley kernel yield. Within the region of the marker, there are 22 genes (Supplementary Table 4). Of these, two have putative functions of interest; a Leucine-rich repeat receptor-like protein, and a WRKY DNA-binding protein (Table 1)—the latter of which has the marker located in it. These account for 301 (0.4%) and 77 (0.1%) genes genome wide, respectively.

Genome-wide association mapping for the mean isolate data (Supplementary Fig. 6) identified no peaks above the threshold set, with the only areas showing indications of regions of interest being at 2HL for lesion area (a) and 5HL for infection lesion severity (b). The GWA mapping for the isolates 13–13 (Supplementary Fig. 7) and L73A (Supplementary Fig. 8) also showed no significance but with

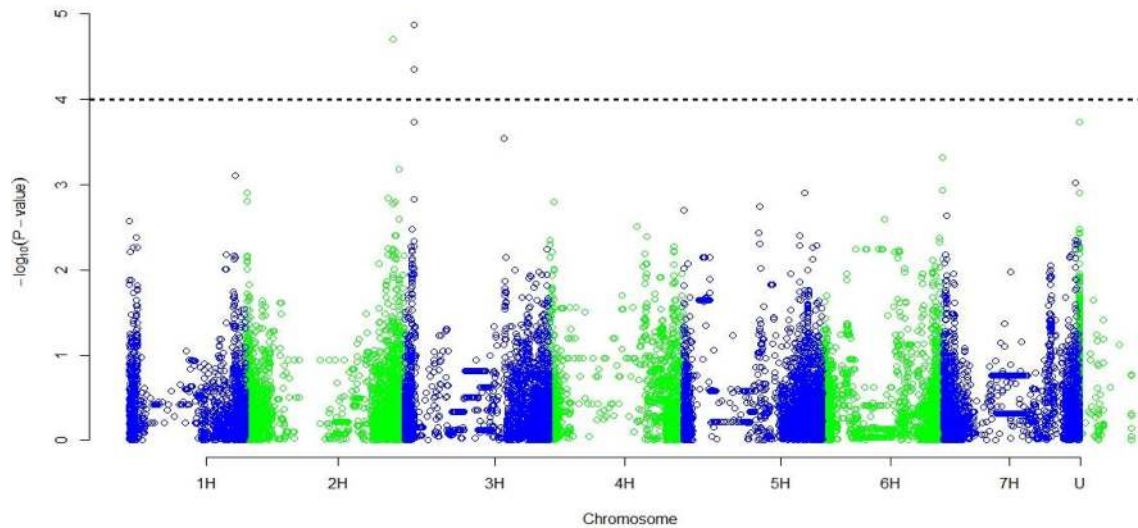
indications at chromosome 7HS in the 13–13 lesion area, and 3HL in the L73A lesion severity data.

Genome-wide association mapping of the field trial data showed one significant association on the distal end of chromosome 3HS in the region of 44.46–45.33 Mb (Fig. 6) with eight significant markers. Within this region, there were 45 genes (Supplementary Table 4), of which only one had a putative function that is known to relate to pathogen infection, a cysteine-rich receptor-like protein kinase with a domain homologous to Gnk2 mentioned above (Table 1). One significant marker, with the largest statistical effect, is found on chromosome 2HL at 713.11 Mb (not in the same area as found the DLA data) with 40 genes in the region (Supplementary Table 4). Within this region, there are four genes with a putative protein annotation of interest, including a cysteine-rich receptor-like protein kinase with a domain homologous to Gnk2 as mentioned above, a Glycosyltransferase family protein, and two disease-resistance proteins with leucine-rich repeats—one of the latter of which has the marker within (Table 1).

## Discussion

Barley scald caused by the fungal pathogen *Rhynchosporium commune* is a disease of global significance, and a major problem in most parts of the UK (Avrova and Knogge 2012). It is important to find new sources of resistance, as resistance genes cause a selective pressure on the pathogen to develop





**Fig. 6** A Manhattan plot of a Genome-Wide Association Study undertaken using a mixed linear model approach on relative area under disease progress curve (RAUDPC) of rhynchosporium during a 2017 field trial; data generated using an ANOVA. Depressions in

marker significance observed in the centre of each chromosome are due to reduced marker density around the centromere of the physical map. The dotted line denotes a significance threshold of a  $p$  value  $< 0.0001$

a mechanism to break the resistance of the plant (Bergelson et al. 2001). Resistance genes that have developed in discrete populations, such as landraces, pose a potential source of novel resistance genes that can be bred into the elite cultivars (Silvar et al. 2010). In this study, we assessed landrace lines for rhynchosporium symptoms in both detached leaf assays and field trials. This identified a number of Bere lines that showed signs of resistance, as well as regions within their genome that correlated with reduction in disease symptoms and a number of genes with putative functions associated with disease resistance.

The detached leaf assays performed on the JHI-SBLC showed that there was a range of infection for all sub-categories (Bere, elite cultivars, other landraces/heritage cultivars), with overall the Bere lines showing smaller lesions, but with more necrotic tissue in their lesions. The experiment measuring lesion size is comparable to the study from Coulter et al. (2018) that shows identified resistant lines with lesion sizes reduced by 45% compared to the most susceptible line—Morex. The work presented in this paper also identified Morex as one of the most susceptible lines, with the largest lesions, and identified many other lines with similar levels of lesion size reduction as found in the Coulter et al. (2018) study when compared to Morex. The smallest lesions in this study (Bere 55 A 33) were reduced by 78% compared to Morex, with the three lines identified for consistent resistance—Bere 45 A 23, Bere 58 A 36 Eday, and Bere 8-125—reduced by 71–76% compared to Morex. This supports the anecdotal claims of farmers working with Bere populations that they were more resistant to rhynchosporium than current

elite cultivars (Mahon et al. 2016). However, a large difference between how different isolates interact with individual lines was shown, highlighted by the isolate 13-13 that interacts with lines/cultivars in a differential manner from the remainder of the isolates. The difference seen in the lesion severity is possibly due to the differences in reaction/susceptibility to necrosis inducing peptides from the pathogen, as *R. commune* has not been shown to induce a hypersensitive response (Wevelsiep et al. 1991; Hahn et al. 1993).

Currently, there are multiple rhynchosporium resistance genes that are spread across all barley chromosomes, except 5H (Hanemann 2009; Zhang et al. 2020). The identification of new resistance genes that can be introgressed into elite lines could help make a more robust resistance. This study identified eight regions across multiple chromosomes that are associated with one or more of the isolates with regard to lesion area and/or lesion severity. In the DLA work, this included regions that are near the location of previously identified *Rrs* genes, including: two peaks on chromosome 7HS from the isolate 13-13 lesion area data and the L2A lesion severity data that could correlate with *Rrs2* and *Rrs12*, respectively (Abbott et al. 1992; Hanemann 2009), one peak on 4HS from the isolate L2A lesion area data possibly correlating with *Rrs16* (Pickering et al. 2006), and one peak on chromosome 4HL also from the isolate L2A lesion area data close to the estimated region of *Rrs3* (Bjørnstad et al. 2002). The two regions of interest were identified on chromosome 2HL and 5HL, the latter of which has not had an *Rrs* gene identified on that chromosome before and the former has not had an *Rrs* gene identified in that region (Zhan et al.

2008). Additionally, the region identified in 2HL has not had any QTLs associated with rhynchosporium resistance (Zhang et al. 2020). However, Büttner et al. (2020) have recently identified a QTL in a similar location on 5HL as the one found in this study, further suggesting a potential novel source of resistance.

Within the regions on chromosome 5HL in the DLA data, there were 11 proteins with six different putative functions that were identified as potentially responsible for the reduction in symptoms. These included: (1) zinc finger family proteins—one zinc finger BED domain and three RING/U-box superfamily proteins—the former group have been associated with R genes in barley (Gupta et al. 2012), and the latter group being associated with plant defence mechanisms in response to pathogenic fungi (González-Lamothe et al. 2006; Marino et al. 2012). (2) Pathogenesis-related thaumatin superfamily proteins—which have been linked multiple times to resistance of fungal pathogens in cereal crops including barley (Reiss and Horstmann 2001; Anand et al. 2003; Wang et al. 2010; Zhang et al. 2018). (3) Defensin-like proteins—a group of anti-microbial peptides, with anti-fungal properties in plants shown to inhibit pathogen infection (Andersen et al. 2018; Sher Khan et al. 2019). (4) a cathepsin B-like cysteine proteinase—which have been associated with the plant disease-resistance hypersensitive response (Gilroy et al. 2007), though *R. commune* has not been shown to induce a hypersensitive response (Wevelsiep et al. 1991; Hahn et al. 1993). (5) a MATE efflux family protein—a group that have been shown to negatively regulate plant disease resistance such as to the wheat pathogen *Blumeria graminis* f. sp. *tritici* (Sun et al. 2011). (6) a receptor kinase with a domain homologous to Ginkbilobin-2 (Gnk2)—noted as Gnk2 is an antifungal protein found in *Ginkgo biloba* causing the inhibition of the growth of fungal pathogens such as *Fusarium oxysporum* (Miyakawa et al. 2014).

The marker identified on chromosome 2HL was located within a gene with the putative function of a WRKY DNA-binding protein that have been shown to be involved in the defence against both biotic and abiotic stresses (Agarwal et al. 2011; Zhu et al. 2013) such as the suggested response to powdery mildew in barley (Wang et al. 2014). Within the region, a gene with a Leucine-rich repeat receptor-like protein putative function was also considered due to its noted contribution to fungal resistance such as to *Fusarium graminearum* in cereals (Thapa et al. 2018) and have been noted as a main class of R gene (Hammond-Kosack and Jones 1997).

The field trial results corroborated that Bere lines on average showed diminished symptoms. The spread of the lines/cultivars shows that there were large amounts of variation

within each sub-category with regard to resistance to rhynchosporium, with individual Bere and other landrace lines being identified in both years as having both small and large levels of disease. Whilst visual scores were not directly numerically comparable to scores done by others, they can still be generally compared. A similar experiment, from Looseley et al. (2018), undertook trials in the same fields over three years with common elite cultivars to assess European spring barley germplasm. As in this study, Propino consistently showed amongst the smallest infection of the common elite cultivars, though in contrast to this experiment so did Belgravia. The most resistant lines from each year of the Looseley et al. (2018) trial showed up to a 54% reduction in their AUDPC compared to Propino. In contrast, the most resistant line in the 2017 trial showed a reduction of 84% compared to Propino, with the three lines identified for consistent resistance mentioned above showing 67–83% reduction compared to Propino. This variation could be due, in part, to the different environmental conditions found between the origins of the lines, with some coming from heterogeneous populations (Ceccarelli et al. 1987; Yitbarek et al. 1998). However, the elite cultivars showed comparatively much greater levels of infection than the DLAs suggest. This could be explained by the difference in height, as the effect of the dwarfing genes in elite lines gives a smaller distance between the leaf nodes making it more likely for the spores to spread by splash up onto higher leaves (von Korff et al. 2005; Looseley et al. 2012). The results also showed that there was no correlation between the lines grown in 2016 and the same lines in 2017 when comparing infection levels, this is possibly due to different environmental conditions and/or shifts in the *R. commune* population between years in the field (Looseley et al. 2015, 2018). This difference could highlight the need for increased discovery of resistance mechanisms to provide a broad protection against the unpredicted annual changes in epidemiological conditions and pathogen isolates in the same field.

Using the data from the 2017 trial, a QTL on chromosome 3HS was found, significantly associated with differences in symptom along with a significant marker on chromosome 2HL. Neither region has any known *Rrs* gene and thus may be evidence of potential sources of novel resistance genes. The region found in 3HS contained a cysteine-rich receptor-like protein kinase with a domain homologous to Gnk2, noted as above (Miyakawa et al. 2014). The region around the marker on 2HL identified a number of genes of interest based on their putative function including another cysteine-rich receptor-like protein kinase with a domain homologous to Gnk2, two disease-resistance family proteins, and a Glycosyltransferase family protein. The latter of which was selected as this family of protein has been shown to

confer resistance to *Fusarium* in barley (Xing et al. 2017) and wheat (He et al. 2018).

No correlation was found between the DLA and field data, again possibly due to architectural differences of the whole plant influencing the number of spores able to splash up the plant (von Korff et al. 2005; Looseley et al. 2012), or due to differences in environmental conditions. Other possible explanations could be that the isolates found in the nursery were dissimilar to those used in the DLA experiment. However, three Bere lines were identified as having consistently low levels of infection in all experiments: Bere 45 A 23, Bere 58 A 36 Eday, and the previously outlined Bere 8-125. As these lines had low infection in the DLA, it shows that it is more than a plant architecture difference that is causing the low infection scores in the field for these lines. Thus, these lines show potential for novel sources of resistance.

Further analysis is necessary to identify whether the resistance exhibited in these lines is, in part or in whole, caused by the identified chromosomal regions of interest in 2HL, 3HS, and/or 5HL, with emphasis on the candidate genes identified. This could be achieved by creating a biparental mapping population to fine map the regions of interest. If these regions are the cause of this increased resistance, they could continue to be introgressed into elite breeding lines to develop new cultivars. This would help protect the elite crops from rhynchosporium infection by providing an additional form of resistance that the pathogen would need to overcome. The development of cultivars with a robust resistance to all isolates of rhynchosporium is important, especially with the potential of ongoing climate change resulting in differing selective pressures on both the crop and the pathogen, that may promote the evolution of *R. commune* to break current resistance or that may weaken the crop making it more susceptible (Stefansson et al. 2013; Velásquez et al. 2018).

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s41348-021-00470-x>.

**Acknowledgements** This research was funded by the Agriculture and Horticulture Development Board (AHDB), through a Cereals & Oilseeds PhD Studentship, and the James Hutton Institute. We thank Christine A. Hackett (BioSS) for statistical advice, and Amy Learmonth for guidance in the GWAS analysis. The technical assistance of Carla De La Fuente Canto, Trevor Mason, Clare Macaulay, Malcolm Macaulay, Richard Keith, Christopher Warden, and especially David Guy is also greatly appreciated. Final thanks for funding go to the Scottish Government's Rural & Environment Science & Analytical Services (RESAS) for funding associated work by Adrian Newton and Tim George. Experimental work, analysis, and paper writing were undertaken by Jonathan Cope, Gareth Norton, Timothy George, and Adrian Newton provided supervision and advice throughout the project, as well as significant editorial decisions in creating the paper. Raw data is available upon request by contacting the corresponding author. This study was funded by AHDB Cereals & Oilseeds (GB) (Grant Number SR49).

## Declaration

**Conflict of interest** The authors declare that they have no conflict of interest.

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