Resistance to *Rhynchosporium commune* in a collection of European spring barley germplasm

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Key message: **Association analyses of resistance to *Rhynchosporium commune* in a collection of European spring barley germplasm detected 17 significant resistance quantitative trait loci. The most significant association was confirmed as *Rrs1*.**

Abstract

Rhynchosporium commune is a fungal pathogen of barley which causes a highly destructive and economically important disease known as rhynchosporium. Genome wide association mapping was used to investigate the genetic control of host resistance to *R. commune* in a collection of predominantly European spring barley accessions. Multi-year disease nursery field trials revealed 8 significant resistance quantitative trait loci (QTL), whilst a separate association mapping analysis using historical data from UK National and Recommended List trials identified 9 significant associations. The most significant association identified in both current and historical data sources, collocated with the known position of the major resistance gene *Rrs1*. Seedling assays with *R. commune* single spore isolates expressing the corresponding avirulence protein NIP1 confirmed that this locus is *Rrs1*. These results highlight the significant and continuing contribution of *Rrs1* to host resistance in current elite spring barley germplasm. Varietal height was shown to be negatively correlated with disease severity, and a resistance QTL was identified that co-localised with the semi-dwarfing gene *sdw1,* previously shown to contribute to disease escape. The remaining QTL represent novel resistances that are present within European spring barley accessions. Associated markers to *Rrs1* and other resistance loci, identified in this study, represent a set of tools that can be exploited by breeders for the sustainable deployment of varietal resistance in new cultivars.

Keywords: barley, rhynchosporium, resistance, *Rrs1*, QTL, GWAS

Introduction

Barley (*Hordeum vulgare* L.) is the fourth most widely grown cereal crop, that was cultivated on over 49 million hectares worldwide and produced 141 million tonnes of grain in 2016 (faostat.fao.org). Whilst most barley is used as a carbohydrate source in animal feed, approximately 20% of worldwide production is processed. The majority of this is used for malting in order to produce alcoholic drinks.

Fungal pathogens represent the main constraint to barley production, with the fungal pathogen *Rhynchosporium commune* causing one of the most economically significant and destructive diseases of barley worldwide (reviewed in Avrova and Knogge 2012). This disease is known as rhynchosporium, barley scald, or leaf blotch. Whilst its primary significance is through severe decreases in yield, with losses of up to 40% when conditions are favourable for disease development (Xi et al. 2000), it can also affect grain quality traits through a reduction in grain size (Khan and Crosbie 1988) leading to increased grain nitrogen content and screenings (the proportion of grains passing through a set sieve size). *R. commune* has been classified as a hemibiotroph (Perfect and Green 2001; Oliver and Ipcho 2004); despite producing necrotic lesions, it has a long asymptomatic phase during which it is able to colonise the subcuticular region of the epidermis and even sporulate (Zhan et al. 2008; Thirugnanasambandam et al. 2011; Avrova and Knogge 2012). *R. commune* is a polycyclic pathogen, with primary inoculum coming either from crop debris or infected seed (Davis and Fitt 1992; Fitt et al. 2010). Agronomic practices such as seed treatment, crop rotation, tillage and grazing are important ways of controlling the occurrence of the disease, by limiting primary inoculum (Arvidsson 1998; Elen 2002). Secondary inoculum is formed by conidia produced on infected leaves, which spread infection up the plant by splash dispersal (Fitt et al. 1988). The primary method of disease control in the field is through fungicide application. However, *R. commune* is a highly genetically diverse pathogen (Zaffarano et al. 2006) and has developed insensitivity to previously effective fungicide classes, e.g. methyl benzimidazole carbamates and demethylation inhibitors (Taggart et al. 1999; Avrova and Knogge 2012).

Varietal resistance is another effective way of providing protection against initial infection and is an important and sustainable method of disease control. Major resistance (*R*) genes trigger plant defence responses by directly or indirectly recognising the products of avirulence genes expressed by the pathogen during infection. However, due to the simple genetic architecture of this interaction, major gene mediated resistance can be broken down

after only a short period of commercial cultivation (Newton et al. 2001; Abang et al. 2006), unless the avirulence gene product is essential to the pathogen. An example of this is the mutation or loss of the avirulence gene *NIP1*, under the strong selective pressure of *Rrs1*-carrying cultivars which are able to recognise NIP1 (Rohe et al. 1995; van't Slot et al. 2007).

A number of studies have reported partial resistance that reduces rhynchosporium severity (Williams and Owen 1975; Xue and Hall 1991; Kari and Griffiths 1993; Schweizer and Stein 2011; Looseley et al. 2012). As partial resistance relies on less specific interactions with the pathogen, it is likely to be more durable (Poland et al. 2009), but the limited magnitude of the effect of partial resistance genes means that they are unlikely to provide sufficient levels of varietal resistance if used in isolation. Increasing the effectiveness of these various crop protection tools is likely to be achieved through adopting an integrated approach to disease management, using a combination of fungicides, agronomic practices and varietal resistance. The use of resistant cultivars carrying polygenic, and therefore more durable, resistance (both *R* genes and quantitative resistance) with complementary effects is the most sustainable and cost effective method of protecting the considerable breeding effort required to identify and incorporate resistance genes into elite cultivars (Walters et al. 2012). However, due to the difficulty of distinguishing between the effects of alternative resistance genes (particularly with epistasis) the generation of polygenic resistance is problematic for commercial breeders using only phenotypic selection, and as such, there is a requirement, not only for new sources of resistance, but also for the identification of closely linked, or diagnostic markers for marker assisted breeding.

Several major resistance genes and quantitative trait loci (QTL) against *R. commune* have already been mapped. *Rrs1* on 3H (Hofmann et al. 2013), *Rrs2* on 7H (Hanemann et al. 2009), *Rrs4* on 3H (Patil et al. 2003) and *Rrs15b* on 2H (Schweizer et al. 2004) originated from *Hordeum vulgare*, but wild *Hordeum* species have also been used as a source of resistance. *Rrs12* on 7H (Abbott et al. 1992), *Rrs13* on 6H (Abbott et al. 1995), *Rrs14* on 1H (Garvin et al. 2000) and *Rrs15a* on 7H (Genger et al. 2003; Genger et al. 2005) were first described in crosses with resistant *H. spontaneum* accessions, while *Rrs16* on 4H (Pickering et al. 2006) was introduced from *H. bulbosum*. So far, none of these genes have been cloned.

The first resistance locus mapped was *Rrs1* on 3H (Backes et al. 1995; Thomas et al. 1995) and to date more than 11 alleles have been described (Bjørnstad et al. 2002; Hofmann et al. 2013) leading to a debate over whether *Rrs1* is a complex locus comprising multiple tightly linked genes, or different alleles of the same *R* gene. The functional effect of *Rrs1* seems to be

the prevention of penetration and subcuticular growth (Lehnackers and Knogge 1990; Carisse et al. 2000; Thirugnanasambandam et al. 2011) of *R. commune* isolates carrying avirulent allele of *NIP1* (Rohe et al. 1995). NIP1 is an avirulence protein, which does not trigger the hypersensitive response (HR) during plant pathogen interaction (Hahn et al. 1993). It has been shown to interact with the barley plasma membrane H^+-ATPase independently of the barley genotype suggesting that at least one extra genotypically dependant mechanism is involved in activating the resistance, such as another protein or a conformational change of the target, induced by NIP1 interaction with plasma membrane (van't Slot et al. 2007).

Genetic and genomic resources for barley have developed rapidly over recent years, with simple and effective genotyping platforms available at a variety of scales (Moragues et al. 2010; Comadran et al. 2012). In addition, a high quality reference genome assembly has now been made available (Mascher et al. 2017). These resources have allowed Genome Wide Association Studies (GWAS) to be used for identifying loci affecting quantitative traits in barley with the potential to identify candidate genes (Cockram et al. 2008; Comadran et al. 2012). GWAS studies allow significant genetic diversity to be sampled in a single experiment, as well as providing high resolution QTL information (Waugh et al. 2014), and have successfully been used in barley to identify associations with resistance to *Fusarium* head blight, net form net blotch, spot form net blotch, stem rust, spot blotch, and leaf rust (Massman et al. 2011; Zhou et al. 2014; Ziems et al. 2014; Tamang et al. 2015; Richards et al. 2017).

The aim of the current study was to identify and map QTL influencing resistance to *R. commune* in cultivated north-western (NW) European spring barley. In order to identify robust associations, a complementary set of contemporary and historical field trial data were used to validate marker associations and to identify field resistances that retain effectiveness against current pathogen populations. A further aim was to use single isolate teste to validate highly significant associations and characterise these resistance effects. Markers associated with QTL identified in this study will allow cost-effective improvements in resistance against this important barley pathogen.

Materials and methods

Disease nursery trials

A collection consisting of 660 lines of spring barley was tested in field trials at the rhynchosporium disease nursery at the James Hutton Institute's Invergowrie site near Dundee

in Scotland. This collection was collated from lines included in the Biotechnology and Biological Sciences Research Council (BBSRC) funded IMPROMALT project BB/K008188/1, and the ERA-PG-funded project ExBarDiv (Xu et al. 2018), and predominantly represented diversity across current and historical NW European spring barley accessions. Disease assessments were conducted over the course of three growing seasons (2013-15) with the majority of lines (73%) present in all three trials, and a large majority (95%) present in at least two trials. For each of the trials, two replicates were sown using a randomized row and column design. Trials were sown as either 1.5m^2, or 3m^2 plots using a sowing rate of either 120 or 333 seeds/m^2 respectively (Table S1). Continuous growing of barley in the disease nursery had resulted in considerable build-up of inoculum so that natural infection occurred and was encouraged by application of overhead irrigation on alternate days. Visible disease symptoms were assessed according to the method described by Looseley et al (2015). Briefly, plots were scored on a 1-9 scale, where 1 represented complete absence of disease symptoms and 9 a complete coverage of the non-senescent leaf area by lesions. Disease symptoms were assessed 2-3 times per season. In the 2014 and 2015 trials, average height to the base of the ear was measured after stem extension had ceased. A standardised area under the disease progress curve (AUDPC) of each plot was calculated for all trials (Simko and Piepho 2012). Details of trials and timing of disease and height assessments are provided in Table S1.

For each trial, line means were estimated using GenStat 18 software (VSN International 2011) by comparing different REML mixed models. In each case, the fixed model comprised the barley line, and the random model included replicate. For more complex models, additional terms accounting for spatial effect were added to the random model. These included row and column effects as well as a residual term accounting for their interaction. The VSTRUCTURE procedure was used to specify a correlation model for the spatial terms using either a 1st order autocorrelation or identity structure. REML models were compared using a likelihood ratio test to compare the effects of adding in row and column effects in all combinations to the basic randomised complete block model: the simplest model for which there were no significantly better models was used to estimate line means.

Estimated means from each year were standardised following the formula $Z=(x-\mu)/\sigma$ where x is the estimated mean of the line, μ is the mean of the population and σ is the standard deviation of the population.

Shapiro–Wilk tests for normality testing, correlation test and one way analysis of variance were run using GenStat 18 software (VSN International 2011).

Historical disease scores

In the UK, value for cultivation and use (VCU) is assessed for new cultivars prior to inclusion on the National List (NL) in a series of trials coordinated by the British Society of Plant Breeders (BSPB). The best of these lines are then entered into the Agriculture and Horticulture Development Board (AHDB) Cereals and Oilseeds division Recommended List (RL) trials, the results of which are used by AHDB to recommend cultivars to grow to farmers. Rhynchosporium disease severity is assessed as percentage disease cover of the upper leaves due to natural infection in trials that have not been treated with fungicides as part of both the NL and RL trial protocols. The rhynchosporium disease scores for the period 1990-2014 were collated from the NL and RL trials as part of the IMPROMALT project BB/K008188/1. Cultivars included in this data set were present in these trials for a variable number of years (mean 3.9 years, range 1-23 years). For each year, data was collected from between 4 and 22 trial sites. Best Linear Unbiased Predictors (BLUPs) of the means for each cultivar were calculated using the REML directive in Genstat 18, using a random model consisting of site (nested within year); trial series (RL or NL); genotype; genotype by year interaction; and genotype by site interaction. The final data set consisted of BLUPs for 364 cultivars (Table S2).

Genotypes and genetic map

Genotypes for a subset of the lines, for which phenotypic data was available, were generated using the 9k barley iSelect SNP genotyping platform (Comadran et al. 2012). This comprised a total of 595 lines of which 364 had historical phenotypic data, and 499 had disease nursery scores. Of the genotyped lines, 301 had both historical and disease nursery phenotypic data (Table S2). Within each subset, SNPs with greater than 20% missing, together with those having a minor allele frequency of less than 10% were excluded from further analysis in order to provide robust marker trait associations. The final marker set used for the GWAS comprised 4580 SNP markers for disease nursery trials, and 4377 SNP markers for the historical data set. The R package, LPmerge, was used to merge IBSC, PopSeq and BOPA maps (Muñoz-Amatriaín et al. 2011; Mayer et al. 2012; Ariyadasa et al. 2014) into a single consensus map (Xu et al. 2018).

GWAS

For lines with 9k genotypes and disease nursery scores, multiple environment association analyses, treating years as environments, were performed in GenStat 18 using the QMASSOCIATION procedure. Population structure was accounted for using an Eigen analysis, with significant PCA scores being included as random term. The first two principal components from an overall analysis of the 601 lines, which had both genotypes and phenotypes, are shown in Figure S1. The VCMODEL option was used to select the best variance/covariance matrix model for environments (years) according to the Schwarz information criterion, this was the compound symmetry model in each case, indicating that variances and covariances were correlated across years.

For lines with 9k genotypes and RL/NL mean rhynchosporium scores, a single environment association analysis was conducted using the QSASSOCIATION procedure of Genstat 18, again using an Eigen analysis to correct for population structure, with significant PCA scores being included as a random model term.

QTL identification

For the analysis using historical phenotypic data and a single environment GWAS, the effective marker matrix dimensions were used to derive a significance threshold ($-\log_{10}p$) of 3.2 for a genome wide significance level of 0.05 using the 'THRMETHOD' option of the QSASSOCIATION procedure. The same absolute threshold was used for the multi-environment GWAS of AUDPC scores, but was increased to 5 for the height scan to reflect the higher median inflation factor for this trait, and in order to restrict candidate QTL to only the most significant associations. Associated markers were considered as part of distinct QTL if sets of markers with significances greater than the threshold were separated by an interval greater than +/-10 cM from a peak marker as described by Tondelli et al. (2013).

Location of previously reported resistance genes

Information about previously mapped major resistance genes, and other genes reported to affect *R. commune* resistance, including their flanking markers was collated from the literature. The flanking markers were used to locate the major resistance genes on the iSelect map used in this study. For studies that used markers that were not represented on the iSelect map, marker or primer sequences were used in a BLASTn search with default settings against the Morex reference assembly (Mascher et al. 2017). These positions were used to identify flanking

iSelect markers with known physical positions, allowing genetic intervals to be identified for the current map.

For QTL associated with previously reported major genes (where the published interval was less than 25cM), historical trends in QTL were investigated by comparing allele frequencies for peak markers for QTL against the year for which cultivars were first entered for UK NL trials. Cultivars were divided into 8 sets, grouped by date of introduction, and the frequency of the allele associated with the resistant phenotype of the peak QTL marker calculated.

Single spore isolate tests

Seedling resistance screens with *R. commune* isolates avirulent on barley lines containing *Rrs1* were performed. Multiple isolates were used as most isolates are avirulent on multiple major resistance genes. These screens used spray inoculation and visible disease scoring with single spore isolates LfL12F and R214, and a detached leaf assay with *R. commune* strain T-R214-GFP, a green fluorescent protein (GFP) expressing version of isolate R214, (Thirugnanasambandam et al. 2011) in combination with confocal microscopy.

A seedling spray inoculation assay using *R. commune* isolate LfL12F (avirulent on *Rrs1, Rrs2* and *Rrs13*) was conducted as described in Schweizer et al. (1995) with modifications to assess symptoms development. Briefly, four seeds per test line were sown in 9x9 cm pots. Pots were kept at 18°C with 16 h light per day. Three weeks after sowing (late 3 leaf stage (DGS 13-14) (Tottman 1987)), plants were spray inoculated with a spore suspension (2 x 10^5 spores/ml) and kept at 16°C in the dark at 100% humidity for 48 h. Subsequently, plants were kept at 16°C with 16 h light. Symptoms were assessed on a 0-4 scale with 0 representing no visible symptoms, 1 for very small lesions on edge and tip of leaf, 2 for small defined lesions on edge and basis of leaf, 3 for big, confluent lesions on the whole leaf and 4 for total collapse and drying-out of the leaf (Figure 1) (Jackson and Webster 1976). 66 European spring barley lines, predicted as carrying an allele conferring resistance (18 lines) or susceptibility (48 lines) at the *Rrs1* locus, based on results from the GWAS analysis, were screened, supplemented by: 2 Spanish landraces SBCC154 and SBCC145, carrying $Rrs1_{Rh4}$ (Hofmann et al. 2013); spring barley cultivar Pewter, carrying *Rrs2* (Hanemann et al. 2009), and the winter barley cultivar Retriever, likely to be carrying *Rrs1* (Looseley et al. 2015). Scores at 16 days post inoculation (dpi) were used to determine resistance or susceptibility. Overall scores were recorded for each of the four seedlings and means calculated for each line. Lines with mean score of 2 and higher

were considered susceptible. For 4 of these lines, a 2nd seedling assay using isolate R214 (virulent on *Rrs2*, but not on *Rrs1*) was conducted according to the same protocol.

For the detached leaf assay 3-5 plants of each selected line were grown for 2-3 weeks until the emergence of the 3rd leaf in a glasshouse at 17ºC under 16 h day length. Detached leaf assays were performed as described in Newton et al. (2001). Briefly, rectangular polystyrene boxes (79 x 47 x 22 mm) (Stewart Solutions) were filled with approximately 20 ml of 0.5 % water agar with 0.8 mM benzimidazole (Sigma). Five 4 cm leaf segments were placed with the abaxial surface onto the set agar in each box. Leaves were brushed using a sable hair paintbrush to remove some of the cuticle waxes, to prevent water droplets sliding off the leaf surface. The abraded area of each leaf was inoculated with 10 µl of spore suspension adjusted to 10^4 spores/ml and the boxes incubated in a controlled environment cabinet (Leec, model LT1201) at 17ºC under 16 h day length. Confocal imaging of 3-5 inoculation spots was performed at 2-3 dpi as described in Thirugnanasambandam et al. (2011) on a Leica SP2 confocal microscope using an excitation wavelength of 488 nm. GFP fluorescence was imaged between 505 and 530 nm. Overall 28 European spring barley cultivars predicted to have an allele conferring either resistance (14 lines) or susceptibility (14 lines) at the *Rrs1* locus, based on results from the GWAS analysis, were screened, supplemented by the same lines (apart from Pewter) that were added to the spray inoculation assay above. Lines were qualitatively differentiated as resistant, with a restricted randomised mycelial growth, and susceptible, with extensive mycelial network outlining barley epidermal cell walls (Looseley et al. 2015). Representative images of resistant and susceptible interactions are shown in Figure 1.

Reactions against single spore isolates differential against *Rrs2* were taken from the AGOUEB project final report (Thomas et al. 2014) in order to compare these results against a QTL identified next to the *Rrs2* interval.

Results

Phenotypes

Rhynchosporium infection occurred in each of the years over which barley accessions were tested in the disease nursery trials, with a normal distribution of AUDPC observed in all 3 years (Figure 2A-C). From the REML analysis for disease nursery trials, the effect of genotype was highly significant ($p<0.001$) in all years and for all traits. Interestingly the distribution of mean

disease scores of the cultivars from RL/NL was skewed towards lower levels of rhynchosporium (Figure 2D), which may reflect the use of minimum standards of disease resistance in the recommendation of cultivars to UK growers by AHDB Cereals & Oilseeds. Correlation coefficients between disease severity estimates for cultivars from RL/NL trial data and disease nursery rhynchosporium severity scores were moderate but highly significant for all disease nursery trials (Table 1).

A wide variation in mean height, ranging from 70 to 170 cm in 2014 and from 50 to 130 cm in 2015, was observed in the collection of spring barley accessions tested in the disease nursery trials (Figure 2E-F). Overall 2.4- and 2.6-fold variation for plants height was recorded in this collection of barley accessions. The height distribution was skewed towards lower height in both years (Figure 2E-F). Mean barley accession height (measured in the disease nursery trials) was significantly correlated across years and showed a moderate to weak negative correlation with all measures of rhynchosporium severity (Table 1).

Marker-trait associations

Associations between markers and disease severity were identified on all but two (1H and 5H) of the seven barley chromosomes from disease nursery trials (Figure 3A, Table 2). Overall, 8 QTL were identified showing significant associations with AUDPC in the disease nursery trials, 3 of these QTL are located on chromosome 3H (Table 2). Out of the 8 QTL, 4 showed evidence for a QTL x environment (GxE) interaction, but in no case was there evidence of significant cross-over interactions.

The single environment GWAS analysis identified 9 QTL for historical disease scores from RL/NL trials. These were located on barley chromosomes 3H, 4H, 5H and 7H (Figure 3B, Table 3).

In addition, 6 QTL were identified which showed significant associations with height in the 2014 and 2015 disease nursery trials (Figure 3C, Table 2). Three of the height QTL showed significant interactions with year (environment) but there was no evidence for significant cross-over effects for any of these.

Genomic inflation was observed in each of the GWAS analyses although this was of moderate magnitude for rhynchosporium assessments (Figure 3D–E). For the analysis of height, the observed genomic inflation was substantially higher (Figure 3F).

For both data sources, the most significant disease severity association (QA3 and QI2) was with marker SCRI_RS_221644, located at 53.5 cM on chromosome 3H with -$\log_{10}$p scores

of 9.9 and 6.3 respectively (Figure 3A-B, Table 2, Table 3). The minor allele frequencies (MAFs) for this marker were 12 % and 17 % from disease nursery and RL/NL data respectively (Table 2, Table 3). QA3 and QI2 were responsible for the largest effects on disease severity in all three years of disease nursery trials and historical disease scores from RL/NL trials (Table 2, Table 3).

From the previously published studies, flanking markers for nine major resistance genes against rhynchosporium were placed on the current genetic map, along with the semi-dwarfing gene *sdw1* (Figure 3A-C, Table 4). In most cases the map interval for these loci was less than 15 cM, although in the case of *Rrs14* and *Rrs16*, it was approximately 20 cM, and, in the case of *Rrs13*, 30 cM. A number of the map intervals for these resistance loci overlapped with QTL identified in the current study. The *Rrs1* interval on chromosome 3H between 48.7 and 59.6 cM coincided with the most significant rhynchosporium resistance QTL identified from both the RL/NL means and from the disease nursery trials (Figure 3A-B, Table 2-4).

No QTL within the published interval of *Rrs3* was detected in disease nursery trials, but the map interval of QI5 on chromosome 4H overlapped with the published *Rrs3* interval, although it was an effect of the major allele with 59 % of cultivars containing the marker allele associated with resistance (Figure 3A-B, Table 3, Table 4).

The map interval for *Rrs13*, located on the short arm of chromosome 6H included QA7, although the published interval of this gene spanned a large interval of 18.8 Mb (Figure 3A, Table 2, Table 4). QA7 showed inconsistent effect over years with the strongest resistance effect, contributed by the major allele, in 2015 (Table 2). It is not clear from these results whether the QTL detected represented an effect of *Rrs13.* The presence of the allele associated with resistance at high frequency since the 1970s suggests that this is not the case (Table 5).

The physical map interval of *Rrs15b* on chromosome 2H included the peak marker for QA1 which had a fairly low, inconsistent effect over years with the strongest effect in 2013 (Figure 3A, Table 2, Table 4)

The published interval of *Rrs16* spanning ~19 cM equivalent to ~11 Mb at the start of 4H coincided with QA6, detected in the disease nursery trials, which only had a significant effect on disease severity in 2015 (Figure 3A, Table 2, Table 4). In the case of QA6, the major allele was associated with resistance, with 86 % of cultivars containing the marker allele associated with resistance (Table 2). The frequency of the peak marker allele associated with the resistance at QA6 increased from 33 % in lines entered for NL trials in 1970-1980 to over

50 % by 2000-2005 (Table 5). Such a high frequency yet again makes it unlikely that this resistance represents an effect of *Rrs16* introduced from *H. bulbosum.*

QI8 was located close to the published position of *Rrs2*. To examine whether QI8 was an effect of *Rrs2*, results from a differential isolate screen reported in Thomas et al (2014) were tested against iSelect 9k markers from the region of 7H. Whilst the peak QTL marker, 11_21419, was weakly associated with *Rrs2* resistance (Fisher's exact test: p=0.06), a second marker, 11_20242, on the other side of *Rrs2* interval, that was not significant in the current GWAS (that identified QI8), showed a substantially stronger association with the data from Thomas et al (2014) (Fisher's exact test: p<0.0001) suggesting that QI8 was not an effect of *Rrs2* resistance.

The most significant association with height, QH4, (as measured in the 2014/2015 disease nursery trials) coincided with the published map interval of the semi-dwarfing gene *sdw1* (Figure 3C, Table 2, Table 4). This effect also coincided with a significant QTL for disease severity, QA5, identified in the disease nursery trials, with the allele associated with tallness also being associated with a consistent reduction in disease severity over years (Figure 3A, Table 2, Table 4). Similarly, a height effect at 47.2 cM on chromosome 3H, QH3, was coincidental with an effect on AUDPC, QA3, (although not the peak marker) with the allele associated with tallness also being associated with a consistent reduction in disease over three years of disease nursery trials (Figure 3A, C, Table 2).

In general there was a tendency for resistance associated alleles at peak markers for QTL co-localising with known resistance genes to increase in frequency over time, although marker SCRI_RS_138723, associated with *sdw1* became fixed for the allele associated with the semi-dwarf (susceptible) phenotype in lines recently entered in NL trials (Table 5). The marker associated with the major resistance gene *Rrs1* showed a considerable increase in the frequency of the allele associated with resistance in cultivars released since 2005, becoming the major allele in cultivars released since 2010.

Phenotyping *Rrs1* resistance with *R. commune* isolates expressing NIP1

In order to test whether or not the resistance QTL identified at the mapped position of *Rrs1* was, in fact, *Rrs1*, single-isolate screens using two NIP1 expressing isolates were conducted. A set of 22 barley lines, carrying the resistance associated allele at the peak QTL marker, and 48 barley lines, carrying the susceptibility associated allele at the peak QTL marker, were tested with isolate LfL12F. All but one line with the resistance associated marker allele were resistant

to isolate LfL12F, and 47 out of 48 lines with a susceptibility associated marker allele were susceptible to isolate LfL12F (Table 6). Cultivar Pewter, carrying the susceptibility associated marker allele, but also known to carry *Rrs2* (Hanemann et al. 2009), showed moderate resistance to isolate LfL12F. In addition to barley lines with *Rrs1*, *R. commune* isolate LfL12F is recognised by lines with *Rrs2* and *Rrs13*. Therefore, we also used isolate R214 and T-R214-GFP (a version of isolate R214, expressing GFP) specifically recognised by lines with *Rrs1* but not *Rrs2*, for additional testing of 17 lines with the resistance associated marker allele and 15 lines with the susceptibility associated marker allele, including cultivar Pewter. This showed that Pewter was susceptible to isolate R214 and confirmed the phenotypes obtained with isolate LfL12F for all of the other lines tested (Table 6). The combined data for 70 tested barley lines showed very strong evidence against independence (Fisher's exact test: $p<0.0001$).

Discussion

This study used two separate sources of data, recent disease nursery trials and, historical RL/NL trials, to investigate the genetic basis of resistance to *R. commune* in a collection of predominantly European spring barley accessions. There was a substantial phenotypic correlation between the two data sources (comparable to the phenotypic correlations between years in the disease nursery trials), although only one QTL was common to both data sets. Nevertheless, this shared QTL (within the mapped position of *Rrs1*) showed the biggest consistent effect in both datasets and may largely explain the strength of the phenotypic correlation. There are a number of potential explanations for the differences in the QTL sets that were detected for each data source. One such explanation is that it may represent the effects of variation in pathogen population structure (in time as well as between sites) e.g. (Zhan et al. 2012). This, in itself, may reflect pathogen evolution in response to the widespread incorporation of resistance genes into elite cultivars. Similarly, whilst there was considerable overlap between the varietal sets for each data source (Supplementary Table S2), differences in the composition of each set may affect the ability to detect specific resistance effects due to difference in LD structure or allele frequencies at loci influencing resistance traits.

The consistent and, in some cases, highly significant negative correlation between varietal height and disease severity seen in these experiments supports findings from a number of previous studies that have identified the importance of plant height to field resistance to *R.*

commune, most likely due to disease escape (Fitt et al. 1988; Looseley et al. 2012; Looseley et al. 2015), with the effect not being seen in controlled inoculations (Hofmann et al. 2013). The most significant determinant of height in this study was a QTL at the known position of the well characterised semi-dwarfing gene *sdw1* on chromosome 3H. The peak marker for this QTL (SCRI_RS_138723) also showed a significant consistent association with AUDPC in the disease nursery trials, strongly suggesting that disease resistance represents a negative pleiotropic effect of the height effect at this locus. Similarly, marker 11_10601 at 47.2 cM on chromosome 3H showed a significant consistent association with both AUDPC and height in the disease nursery trials. Whilst this marker was grouped with QA3, the fact that this (rather than the peak marker for QA3) showed an association with height, suggests this may represent a separate effect. Analysis of allele frequency against year of introduction for the marker associated with *sdw1* shows that the allele associated with the semi-dwarf phenotype has become fixed in all lines entered for NL trials since 2005 and has been a minor allele since 1990. This observation likely reflects the improved agronomic performance of semi-dwarf types, but the consequence of this is increased exposure of spring crops to rhynchosporium infection through a reduction in disease escape (Fitt et al. 1988; Looseley et al. 2015), which in turn increases the importance of breeding for resistance in this crop.

The GWAS detected 17 QTL that contributed to field resistance to *R. commune* in the spring barley association mapping panel from both of the GWAS analyses. Five of these QTL locations corresponded to previously reported major resistance genes, *Rrs1*, *Rrs3, Rrs13*, *Rrs15b* and *Rrs16*. Considering that majority of the cultivars used in this study are susceptible to rhynchosporium, it is highly unlikely that the resistant sources used to map these genes have been widely incorporated into spring barley breeding programmes, with the exception of *Rrs1*. It is, however, possible that the QTL represent alternative but much less effective alleles of these resistance loci. The published intervals for all of these resistance genes span from 11 Mb in case of *Rrs15b* and *Rrs16,* equivalent to 7 and 19 cM respectively, to 408 Mb, equivalent to ~15 cM, in case of *Rrs3.* Therefore the QTL detected in this study are likely to represent a novel locus rather than the effect of the major resistance gene. For the disease nursery analysis, QTL effects were often consistent across years, showing no evidence for a QTL by environment interaction. Where significant QTL by environment effects were detected, these appeared to represent an absence of effect in certain years rather than a difference in the direction of the effect. This is consistent with differences in pathogen race structure between years, or an environmental effect on the expression of resistance.

Although the collection of spring barley accessions used in this study contained several cultivars, including, Digger, Livet and Pewter, carrying *Rrs2* (Hanemann et al. 2009), no QTL within the *Rrs2* interval was identified. One of the QTL identified using the historical RL/NL data, QI8, was located on the telomeric region of the short arm of chromosome 7H, very close to the mapped position of *Rrs2*. Nevertheless, the observation that the peak QTL marker showed a weaker association with *Rrs2* phenotypes (taken from a previously published study) than another marker located on the other side of *Rrs2* interval, suggests that the field resistance QTL reported here represents a different effect from the previously reported *Rrs2* resistance. The inability to detect *Rrs2* resistance from either historical data or from disease nursery data might be explained by the absence of markers from *Rrs2* interval, a low frequency of the *Rrs2* allele, or ineffectiveness of *Rrs2* against natural *R. commune* populations following several years of deployment of *Rrs2* in barley breeding, or a combination of these effects.

For both of the phenotypic data sets used to map resistance to *R. commune*, a single marker on chromosome 3H (SCRI_RS_221644) showed the most significant association with disease scores, as well as the largest effect. In each case, the minor allele was associated with the resistant phenotype. The location of this QTL corresponds to the known position of the major resistance gene *Rrs1* (Hofmann et al. 2013). Single isolate tests using NIP1 expressing *R. commune* isolates were consistent with this field resistance representing an effect of *Rrs1*. Cultivar Pewter, with the susceptibility associated marker allele, but also known to carry *Rrs2* (Hanemann et al. 2009), showed moderate resistance to isolate LfL12F. This is consistent with the fact that *Rrs2* is known to recognise isolate LfL12F (Marzin et al. 2016) and when tested with an isolate that is virulent on *Rrs2* (R214), Pewter showed full susceptibility. Although marker SCRI_RS_221644 was quite effective in differentiating between *Rrs1* and non-*Rrs1* barley lines, it is not a truly diagnostic marker, as cultivar Karri had an allele associated with *Rrs1* but was susceptible to *R. commune* isolate LfL12F, expressing NIP1, suggesting an incomplete LD with the phenotype. Further research into characterisation of differential SNPs within *Rrs1* interval is required to identify truly diagnostic markers for *Rrs1*. Taken together, these results are strongly supportive of the interpretation that the field QTL detected here represents an effect of *Rrs1* and supports the observation that *Rrs1* remains effective against natural *R. commune* populations made by Looseley et al. (2015) at the Dundee trial site. This is an interesting observation given that previous studies have demonstrated that *R. commune* has overcome *Rrs1* resistance by losing the expression and/or the function of the recognised form of NIP1 in 45% of the isolates (Schürch et al. 2004). More recently *NIP1* deletion mutants

were shown to cause weaker symptoms on barley cultivars missing *Rrs1* gene suggesting the importance of *NIP1* for virulence (Kirsten et al. 2012). This suggests that *NIP1* expressing isolates might have an advantage over isolates missing *NIP1* in the field population in the absence of a constant selection on *Rrs1* expressing barley cultivars.

A comparison between the frequency of the resistance associated marker allele and the year in which cultivars were first entered into NL trials demonstrates that, whilst the resistance associated allele of marker SCRI_RS_221664 is detectable in cultivars dating back to at least the 1970s, a substantial increase in frequency has occurred since 2005, with the majority of new UK spring barley cultivars now carrying the resistant marker for this locus. This observation likely reflects direct selection for rhynchosporium resistance across this period, although it is not clear whether this is due to phenotypic selection, or from previously published genetic markers. Nevertheless, it is clear that *Rrs1* is present across a variety of current cultivars and as such is highly accessible to UK spring barley breeders. The SNP marker SCRI_RS_221664 that exhibited a high level of LD with *Rrs1* provides a valuable tool for breeders to both introduce resistance into existing breeding programmes and for initial selections.

Similarly, the other associated markers identified in this study, reflect variation that currently exists within UK elite germplasm. This genetic variation represents a resource that can be used in routine marker screening in existing spring barley breeding programmes to increase levels of varietal resistance without the additional problems caused by introgressing resistance from exotic sources. Nevertheless, the ability to detect marker-trait associations using this technique depends on the allele frequency at QTL, and therefore, it is likely that rare resistance genes were not detected by this analysis. The resistance estimates for the barley accessions described in this study are likely to also represent a useful resource for further genetic investigations of resistance in spring barley.

Author contribution statement

MEL, LLG, RW, JR and AA conceived and designed the experiments. HB collated the collection of barley cultivars. PS, MM, and AB produced the genotypic data. MEL, LLG, BB, KMW and JM-W performed the experiments. MEL, LLG and AA analysed the data. MEL, LLG, WTBT and AA wrote the manuscript. All authors read and approved the final manuscript.

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Conflict of Interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Table 1. Phenotypic correlation coefficients between measures of rhynchosporium severity and height. The significance of each correlation coefficient is indicated by asterisks, with a single, double and triple asterisks corresponding to $p<0.05$, $p<0.01$ and $p<0.001$respectively.

Trait	AUDPC			RL/NL Mean	Height 2014
	2013	2014	2015		
AUDPC 2013	-				
AUDPC 2014	0.37 ***	-			
AUDPC 2015	0.53 ***	0.47 ***	-		
RL/NL Mean	0.33 ***	0.32 ***	0.39 ***	-	
Height 2014	-0.24 ***	-0.26 ***	-0.28 ***	-0.14 *	-
Height 2015	-0.15 *	-0.11	-0.14 *	-0.11	0.56 ***

Table 2. Summary of significant marker trait associations identified from a multi-environment GWAS using three years of disease nursery trials. Where multiple associated SNPs (at different positions) were detected for a QTL, the interval over which significant marker associations were identified is indicated. Where effects differ between years, evidence was found for a significant QTL by environment interaction, these are also indicated by an asterisk.

Trait	QTL Name	Chr	Peak Marker	Physical position, bp	Position (Interval), cM	Alleles, Major/ minor	MAF[a]	-log$_{10}$p	Minor allele effect			GxE
									2013	2014	2015	
AUDPC	QA1	2H	SCRI_RS_155957	19,671,074	21.3 (18.6-21.3)	A/G	0.26	3.8	-0.13[b]	0.07	0.10	*
	QA2	2H	SCRI_RS_138045	732,622,007	136.8 (133.3-136.8)	A/G	0.11	3.8	0.02	0.15[b]	0.28[b]	*
	QA3	3H	SCRI_RS_221644	490,226,429	53.5 (47.2-59.6)	A/G	0.12	9.9	-0.57[b]	-0.57[b]	-0.57[b]	
	QA4	3H	SCRI_RS_227898	564,870,387	75.3 (75.3-75.8)	G/A	0.22	4.2	-0.27[b]	-0.27[b]	-0.27[b]	
	QA5	3H	SCRI_RS_138723	632,253,092	112.2 (112.2-118.7)	T/A	0.18	3.2	-0.22[b]	-0.22[b]	-0.22[b]	
	QA6	4H	SCRI_RS_197394	8,808,903	14.3 (14.3-14.3)	T/C	0.14	3.3	-0.03	-0.04	0.19[b]	*
	QA7	6H	SCRI_RS_201251	16,986,968	25.7 (24.2-25.7)	G/A	0.25	3.4	0.05	0.10	0.21[b]	*
	QA8	7H	SCRI_RS_138457	36,912,761	29.7 (18.5-29.9)	A/C	0.49	3.8	-0.19[b]	-0.19[b]	-0.19[b]	
Height	QH1	2H	SCRI_RS_185319	22,770,072	21.8 (15.6-21.8)	G/C	0.15	6.4	-	3.04[b]	1.66[b]	*
	QH2	2H	SCRI_RS_137263	653,415,617	82.3	A/G	0.22	5.1	-	-2.55[b]	-2.55[b]	
	QH3	3H	11_10601	54,950,033	47.2 (47.2-47.4)	A/C	0.28	8.0	-	3.10[b]	3.10[b]	
	QH4	3H	SCRI_RS_138723	632,253,189	112.2 (102.2-123)	T/A	0.18	19.7	-	5.93[b]	3.69[b]	*
	QH5	6H	SCRI_RS_237419	399,378,996	59.1 (59.1-61.9)	A/G	0.41	5.7	-	-0.81	1.89[b]	*
	QH6	7H	11_10209	260,601,407	72 (71.1-72)	G/A	0.47	5.6	-	2.34[b]	2.34[b]	

[a] Minor allele frequency

[b] Indicates that allelic differences were significant within year

Table 3. Summary of significant marker trait associations identified from a single environment GWAS. Where multiple associated SNPs (at different positions) were detected for a QTL, the interval over which significant marker associations were identified is indicated.

Trait	QTL Name	Chr	Peak marker	Physical interval, bp	Genetic position (interval), cM	Alleles, Major/ minor	MAF[a]	-log$_{10}$p	Minor allele effect
RL/NL Mean	QI1	3H	SCRI_RS_162639	22,299,565	30.6	C/T	0.10	3.8	1.11
	QI2	3H	SCRI_RS_221644	490,226,429	53.5 (53.5-61.1)	A/G	0.17	6.3	-1.35
	QI3	3H	SCRI_RS_189322	682,763,236	148.8	T/C	0.30	5.7	0.95
	QI4	4H	SCRI_RS_7704	19,478,534	26.2 (26.2-31.2)	C/T	0.26	3.7	0.83
	QI5	4H	11_20289	462,325,035	50 (48.7-52)	G/A	0.41	4.2	0.73
	QI6	5H	SCRI_RS_204275	543,386,913	78.3	C/A	0.32	3.3	0.63
	QI7	5H	SCRI_RS_235443	568,905,012	96.9 (96.8-96.9)	C/T	0.38	3.8	0.71
	QI8	7H	11_21419	737,055	0.0	G/A	0.43	3.8	0.67
	QI9	7H	12_20832	628,962,539	117.3 (108.8-122)	C/G	0.23	4.3	0.84

[a] Minor allele frequency

Table 4. Location of previously reported genes or loci influencing resistance to *Rhynchosporium commune*.

Gene	Reference	Reference flanking marker(s)	Flanking 9k markers	Chr	Physical interval, bp	Interval, cM
Rrs1	(Hofmann et al. 2013)	11_0010 - 11_0823	12_30609-11_11401	3H	489,991,522-491,895,585	48.7-59.6
Rrs2	(Hanemann et al. 2009)	Acri_SNP9 - 668A17_e11-2_SNP5*	12_20201-12_31350	7H	4,280,866-6,314,541	0.8-1
Rrs3	(Grønnerød et al. 2002)	Hvm003-hvm068	12_11077-11_11513	4H	161,219,174-574,543,534	50.8-65.7
Rrs4	(Patil et al. 2003)	HVM060-WG940	11_20063-12_30090	3H	576,629,513-598,143,391	85.4-96.3
Rrs13	(Abbott et al. 1995)	ABG378-MWG916	11_21032-11_20052	6H	10,327,213-29,107,331	10-40.5
Rrs14	(Yun et al. 2006)	[CHR START]-Bmac0213	12_10420-11_20371	1H	0-12,990,947	0-23
Rrs15a	(Genger et al. 2005)	HVM49	12_20079	7H	647,664,938	136.1
Rrs15b	(Wagner et al. 2008)	GBM1281-GBM1121	11_21377-12_31284	2H	11,188,932-22,398,480	8.5-15.6
Rrs16	(Pickering et al. 2006)	MWG634-scsnp00600	12_31324-11_11136	4H	639,959-11,733,569	0.7-19.6
sdw-1	(Malosetti et al. 2011)	BOPA1_11_10867*	12_11338-12_30096	3H	632,252,063-634,923,676	118.7-119.5

*Diagnostic markers

Table 5. Resistance associated allele proportion compared to year of introduction for QTL associated with known major resistance genes and *sdw1*. In the case of *sdw1*, the frequency of the allele associated with the tall phenotype is indicated.

Marker	Colocalised gene	1970 - 1980	1980 - 1990	1990 - 1995	1995 - 2000	2000 - 2005	2005- 2010	2010- 2014
SCRI_RS_221644	*Rrs1*	0.09	0.06	0.06	0.05	0.08	0.29	0.54
11_20289	*Rrs3*	0.33	0.48	0.57	0.44	0.65	0.66	0.68
SCRI_RS_201251	*Rrs13*	0.56	0.73	0.75	0.81	0.69	0.81	0.78
SCRI_RS_155957	*Rrs15b*	0.17	0.12	0.23	0.17	0.20	0.42	0.68
SCRI_RS_197394	*Rrs16*	0.83	0.76	0.81	0.9	0.93	0.94	0.97
SCRI_RS_138723	*sdw1*	0.83	0.36	0.11	0.09	0.02	0	0

Table 6. Disease reactions of selected barley lines against two *Rhynchosporium commune* isolates, expressing *NIP1*. The name (or identifier) of each line is shown, with text in brackets indicating known major resistance genes carried by the line from published studies. Mean disease scores against isolates LfL12F and R214 are shown, with standard deviation between reps indicated in brackets. For tests of homogeneity, lines with a mean disease score less than 2 were considered resistant. The allele carried at marker SCRI_RS_221644 is indicated (allele 'G' was associated with the resistant phenotype in disease nursery trials).

Line	Disease reaction			SCRI_RS_221644 allele	Seasonal Habit
	LfL12F mean (SD)	R214 Mean (SD)	T-R214-GFP		
SBCC154 (*Rrs1*$_{Rh4}$)	0.0	-	Resistant	G	Spring
SBCC145 (*Rrs1*$_{Rh4}$)	0.0	-	Resistant	G	Spring
Acclaim	0.0	-	Resistant	G	Spring
Beryllium	0.4 (0.4)	-	Resistant	G	Spring
Brahms	0.0	-	Resistant	G	Spring
Cairn	0.0	-	Resistant	G	Spring
Casino	0.3 (0.6)	-	Resistant	G	Spring
Celebra	0.0	-	Resistant	G	Spring
Century	0.0	-	Resistant	G	Spring
Chieftain	0.0	-	Resistant	G	Spring
Corgi	0.0	-	Resistant	G	Spring
Franklin	0.8 (0.3)	-	Resistant	G	Spring
Gairdner	0.0	-	Resistant	G	Spring
Graphic	0.0	-	Resistant	G	Spring
Retriever	0.0	-	Resistant	G	Winter
SW Macsena	0.0	-	Resistant	G	Spring
Westminster	0.0	-	Resistant	G	Spring
Chronicle	0.0	-	-	G	Spring
Freja	0.0	-	-	G	Spring
Magellan	0.0	-	-	G	Spring
Rebecca	0.3 (0.6)	-	-	G	Spring
Karri	3.9 (0.1)	-	-	G	Spring
Acrobat	3.6 (0.7)	-	Susceptible	A	Spring
Alexis	4.0 (0.0)	3.3 (1.3)	Susceptible	A	Spring
Akita	3.4 (0.9)	-	Susceptible	A	Spring
Apex	3.8 (0.2)	-	Susceptible	A	Spring
Ardila	3.5 (0.7)	-	Susceptible	A	Spring
Atlas	2.5 (1.2)	-	Susceptible	A	Spring
Barabas	3.6 (0.1)	-	Susceptible	A	Spring
Beatrix	3.6 (0.5)	4.0 (0.0)	Susceptible	A	Spring
Bulbul 89	2.3 (0.9)	-	Susceptible	A	Spring
Concerto	3.3 (1.0)	-	Susceptible	A	Spring
Gizmo	3.8 (0.3)	-	Susceptible	A	Spring

Imidis	3.1 (0.5)	-	Susceptible	A	Spring
Nordal	4.0 (0.0)	-	Susceptible	A	Spring
Optic	2.2 (0.7)	-	Susceptible	A	Spring
Cropton	4.0 (0.0)	-	-	A	Spring
Aapo	3.3 (0.9)	-	-	A	Spring
Abava	3.2 (0.8)	-	-	A	Spring
Annabel	3.8 (0.5)	-	-	A	Spring
Aspen	3.6 (0.2)	-	-	A	Spring
Atem	3.5 (0.4)	-	-	A	Spring
Athena	3.5 (0.5)	-	-	A	Spring
Azure	3.3 (0.3)	-	-	A	Spring
Baronesse	3.8 (0.2)	-	-	A	Spring
Binder Abed	3.0 (0.4)	-	-	A	Spring
Calgary	2.6 (0.4)	-	-	A	Spring
Chamant	3.7 (0.6)	-	-	A	Spring
Chaser	3.2 (0.3)	-	-	A	Spring
Chevallier Tystofte	3.1 (0.7)	-	-	A	Spring
CPBT_C80	2.9 (1.0)	-	-	A	Spring
Drum	3.6 (0.4)	-	-	A	Spring
Felicitas	3.6 (0.4)	-	-	A	Spring
Frieda	3.2 (0.4)	-	-	A	Spring
Hannchen	3.1 (1.1)	-	-	A	Spring
Harriot	3.4 (0.6)	-	-	A	Spring
Ida	3.9 (0.1)	-	-	A	Spring
Jive	2.5 (0.3)	-	-	A	Spring
Klaxon	3.2 (0.1)	-	-	A	Spring
Kym	3.8 (0.4)	-	-	A	Spring
NSL 95-1257	3.6 (0.5)	-	-	A	Spring
NSL 98_5065	3.9 (0.1)	-	-	A	Spring
A96-103	3.2 (0.7)	-	-	A	Spring
Rangoon	3.8 (0.0)	-	-	A	Spring
Scarlett	2.5 (0.6)	-	-	A	Spring
Steffi	3.6 (0.4)	3.1 (1.2)	-	A	Spring
Tarm 92	3.1 (0.4)	-	-	A	Facultative
Vegas	2.3 (0.3)	-	-	A	Spring
Vortex	3.1 (1.0)	-	-	A	Spring
Pewter (*Rrs2*)	1.6 (0.7)	3.5 (0.0)	-	A	Spring

Figure legends

Figure 1. Representative images showing infection types in both of the controlled environment tests used in this study. The upper two panels show resistant (A) and susceptible (B) interactions as determined by detached leaf assay and confocal microscopy at 2 days post inoculation (dpi) with a GFP expressing *Rhynchosporium commune* isolate (T-R214-GFP). Green colour represents GFP fluorescence and shows fungal spores and hyphae, with blue

colour showing chlorophyll auto-fluorescence. Resistant interactions typically show germinated spores, less extensive hyphal networks, with random growth directions, whilst resistant lines show much more extensive growth following the anticlinal wall of the epidermal cells. The lower panel (C) shows representative leaves illustrating the 0-4 scale used to quantify symptom expression 16 days post-inoculation (dpi) of 3 weeks old barley seedlings with a 2 x 10^5 spores/ml suspension of *R. commune*. 0 represents an absence of visible disease symptoms (not shown) and 4 represents total collapse and drying-out of the entire leaf. Leaves with score of 2 and higher were considered susceptible.

Figure 2. Distribution of phenotypic scores for each of the trait/year combinations examined in this study. AUDPC in disease nursery trials (calculated from disease severity scores on a 1-9 scale) in A: 2013, B: 2014, C: 2015; D: disease severity estimates from historical Recommended List/National List (RL/NL) trial data; height (to the base of the ear after stem elongation had ceased) in disease nursery trials in E: 2014, F: 2015.

Figure 3. Manhattan plots showing association between genetic markers and the traits examined in this study expressed in –log10p. A: multi-environment GWAS using three years of disease severity scores from recent disease nursery trials. B: single-environment GWAS using predicted line means from UK Recommended List/National List (RL/NL) trial data. C: multi-environment GWAS using two years of height scores taken from recent disease nursery trials. Dotted lines indicate the thresholds chosen for selecting putative QTL effects for each trait. For the two disease scores, this is 3.2, representing a genome wide significance level of 0.05. For height, a higher threshold of 5 was used to reflect the higher median inflation factor. The positions of known genes or QTL influencing resistance to *R. commune* are highlighted as light grey bars (indicating an interval), or vertical dashed lines (indicating a position), with names given above the plots. D–F: Observed quantiles of the p-values for each of the GWAS analyses are plotted against their null distribution for datasets in A-C (Q–Q) plots. For each plot, the dashed line represents equality between the observed and expected p-value, and the shaded region represents the 95% confidence interval of the expected values.

Supplementary Data

Table S1. Details of the field trials conducted for the GWAS analyses. For each trial, the dimensions of the plot and sowing rates are indicated along with the date that the trial was sown. The dates are shown for each of the phenotypic assessments.

Table S2. Details of the lines used in the GWAS experiments. The name is indicated along with the AFP code and year that the line was first entered for National List trialling (NL1) where known. AUDPC scores are indicated for each of the disease nursery trials as well as the Recommended List/National List (RL/NL) mean.

Figure S1. A Principal Component Analysis plot of the genotypic data from the 601 genotyped lines used in this study, showing scores for the first two principal components. Figures in brackets following the axis labels indicate the percentage of the total genotypic variation accounted for by the corresponding principal component.