A TaqMan real-time PCR assay for *Rhizoctonia cerealis* and its use in wheat and soil

J.W.Woodhall[1]*, M.J. Brown[1,2], K. Perkins[1,] E. Somoza Valdeolmillos[1] N. Boonham & R.V. Ray[2]

[1]Fera, Sand Hutton, York YO41 1LZ, UK

[2]The University of Nottingham, Sutton Bonington, Leicestershire, LE12 5RD, UK

james.woodhall@fera.co.uk

+44(0)1904 462725

Keywords

Soil DNA, crop rotation, anastomosis group (AG), sharp eyespot,

Abstract

Rhizoctonia cerealis causes sharp eyespot in cereals and the pathogen survives as mycelia or sclerotia in soil. Real-time Polymerase Chain Reaction (qPCR) assays based on TaqMan chemistry are highly suitable for use on DNA extracted from soil. We report here the first qPCR assay for *R. cerealis* using TaqMan primers and a probe based on a unique Sequence Characterised Amplified Region (SCAR). The assay is highly specific and did not amplify DNA from a range of other binucleate *Rhizoctonia* species or isolates of anastomosis groups of *Rhizoctonia solani*. The high sensitivity of the assay was demonstrated in soils using a bulk DNA extraction method where 200 µg sclerotia in 50 g of soil were detected and also the pathogen could be detected in asymptomatic wheat plants. Using the assay on soil samples from fields under different crop rotations, *R. cerealis* was most frequently detected in soils where wheat was grown or soil under pasture. It was detected least frequently in fields where potatoes were grown. This study demonstrates that assays derived from SCAR sequences can produce specific and sensitive qPCR assays.

Introduction

The fungus *Rhizoctonia cerealis* is the causal agent of sharp eyespot in cereals and it is part of the stem-base disease complex. In cereals, sharp eyespot is associated with yield losses due to interference with nutrient and water uptake caused by stem weakening as a result of lesions penetrating the stem (Hamada et al., 2012). The severity of disease caused by the pathogen is thought to have increased during the last two decades (Hamada et al., 2012; Li et al., 2013). This is speculated to be due to climatic changes that could increase the pathogenicity of *R. cerealis* (Hamada et al., 2011). However, Lemańczyk and Kwaśna (2013) highlighted a number of additional reasons including earlier sowing, the use of fungicides that adversely affect microbes that are antagonistic to *R. cerealis*, wider use of susceptible cultivars, transfer of aggressive strains of the pathogen from natural plant communities into crops and the introduction of the pathogen to new growing areas. Since the fungus is primarily soil borne, with infection arising from mycelia within plant debris or germinating sclerotia, a gradual build-up of inoculum in field soils coupled with tighter rotations may also explain the increase in disease severity. Moderate and severe infections can reduce yield substantially (Lemańczyk and Kwaśna, 2013). In the UK, severe infections have been associated with yield losses up to 26% (Clarkson and Cook, 1983). In China, yield losses have ranged from 5 to 40% (Hamada et al, 2011). The fungus has been reported as causing disease in in Europe, North America, Africa, Oceania and Asia (Hamada et al. 2011).

In order to accurately determine losses due to this fungus, correct identification of the causal agent is essential. Sharp eyespot symptoms are often difficult to distinguish due to similarities with symptoms of eyespot (caused by *Oculimacula acuformis* and *O. yallundae*) and foot rot (caused by *Fusarium* and *Microdochium* species) occurring simultaneously on cereal stems (Ray et al., 2004). Rapid and accurate identification of pathogens present in disease complexes are also essential to ensure successful treatment programmes. *Rhizoctonia cerealis* belongs to a species complex consisting of uninucleate *Rhizoctonia*, binucleate *Rhizoctonina* (BNR species) and the multinucleate *Rhizoctonia solani* based on the number of nuclei present in each cell.

Each species is classified to an anastomosis group (AG), the binucleate species are designated AG-A to AG-S whilst multinucleate species designated AG1 to 13 (Sharon et al., 2008). *Rhizoctonia cerealis* is a binucleate species belonging to the AG-D anastomosis group. Whilst hyphal fusion based methods and DNA sequencing offers unequivocal identification of *Rhizoctonia* species to AG or subgroup level, molecular diagnostics methods such as real-time PCR can offer species specific, nucleic acid based rapid detection directly from plant

material or soil (Lees et al., 2002; Okubara et al., 2008; Budge et al., 2009; Woodhall et al., 2013; Boine et al., 2014).

PCR-based methods for identification of *R. cerealis* include conventional PCR assays designed from RAPD fragments (Nicholson and Parry, 1996) and ITS sequences (Chen et al., 2005). Real-time PCR approaches until now have been based on SYBR green chemistry with primers designed to either b-tubulin sequences (Guo et al., 2012) or unique sequences amplified with the microsatellite primer M13 (Hamada et al., 2012). However, previous studies suggest SYBR green assays are strongly influenced by the presence of humic acids which are present in DNA samples extracted from soil (Alaeddini, 2012). Since *R. cerealis* is a soil-borne pathogen, alternative real-time PCR chemistry may be desirable for studies which require detection of the fungus in soil. Real-time PCR assays based on TaqMan chemistry have been shown to be highly suitable with DNA isolated from soil (Ophel-Keller et al., 2008). Therefore we report the development and validation of a sensitive and specific real-time PCR assay based on TaqMan chemistry for *R. cerealis*, its use in plant material and on DNA extracted from a range of UK field soils under different crops. By determining the relative levels of the fungus in soil under different crops, the effectiveness of crop rotations in managing the levels of the pathogen in the soil and providing inoculum in subsequent wheat crops can be determined.

Materials and Methods

Isolates

All isolates used in the study are shown in Table 1. Isolates were obtained from existing culture collections at Fera and the University of Nottingham. Additional isolates were kindly provided by Dr Marc Cubeta, University of North Carolina. Isolates were routinely maintained on potato dextrose agar (PDA) at 20 °C in the dark with longer term storage on frozen barley grains (Sneh et al. 1991). DNA was extracted from 2-3 week old cultures using a Wizard Magnetic DNA Purification System for Food (Promega UK, Southampton) in conjunction with a Kingfisher ML magnetic particle processor (Thermo Scientific UK Ltd) following the manufacturers recommended protocols.

Assay design and real-time PCR

To obtain novel sequence for primer and TaqMan probe sites existing Sequence Characterised Amplified Region (SCAR) primers RC2F and RC2R (Nicholson and Parry, 1996) were used to amplify a putative novel DNA sequence. PCR reactions contained 1 x PCR Master Mix (Fermentas), 400 nM of each primer (MWG Biotech, Germany) and 4 µl template DNA in a total volume of 50 µl. PCR cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 60 s, and a final extension step at 72°C for 5 min. Template DNA used was from isolates RC1, RC2, RC3 and RC4. PCR products were visualised by agarose gel electrophoresis using 2% gels containing 0·5 µg ml^{-1} ethidium bromide in TAE buffer (40 mmol l^{-1} tris-acetate, 1 mmol l^{-1} EDTA, pH 8·0). A QIAquick PCR Purification Kit (Qiagen) was used to purify samples for DNA sequencing which was undertaken by MWG biotech. Resulting DNA sequences were aligned by using ClustalW in Mega5 (Tamara et al., 2011) and Primer Express 3.0 software was used (Life Technologies) to design specific TaqMan® primers and a probe for *R. cerealis*.

Real-time PCR (TaqMan®) was carried out in 96 well plates using the ABI Prism7900HT Sequence Detector System (Applied Biosystems). Environmental Master Mix 2.0 (Applied Biosystems) was used with all samples and consisted of half the total reaction volume of 25 µl. A standard volume of template DNA (5 µl) was used for all samples. Primers and probes (MWG Biotech, Germany) were added to a final concentration of 300 nM and 100 nM respectively with the remaining volume made up with water. Cycling conditions consisted of 50 °C for 2 min, 95 °C for 10 min, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The Ct value for each reaction was assessed using the default threshold ΔRn setting of 0.2 units on the Sequence Detection Software. Each sample was tested in two replicates except for sensitivity testing where three replicates were used. An average (mean) cycle threshold (Ct) was calculated each time. Primer and probe sequences are given in Table 2. Target DNA in soil samples was quantified by including six DNA standards on each PCR run. The standards consisted of a DNA sample of a known concentration taken from a culture of *R. cerealis* which was used to produce a dilution series of five, ten-fold dilutions. The amount of DNA present was then determined by linear regression.

Determining assay sensitivity

A ten-fold dilution series of pure culture DNA from isolate RC1 was used to determine the technical sensitivity of the assay. Sensitivity of the assay in soil was determined by spiking 50 g of sandy-loam soil (determined to be free of *R. cerealis* by real-time PCR) with 0.2 mg to 37.6 mg of purified *R. cerealis* sclerotia (taken from

isolate RC5), a series of 12 spiked samples were prepared. Sclerotia were prepared by growing the isolate on PDA for four weeks in 90-mm petri-dishes and removing them with a scalpel. Sclerotia were then air-dried overnight then macerated using a scalpel, the appropriate weight was added to each soil sample and then left overnight prior to DNA extraction.

Plant material displaying a range of symptoms from healthy to severe were prepared by inoculating 7 day old seedlings of wheat (cv. Gladiator) with sand-maize meal inoculum of *R. cerealis* (isolate RC1) prepared as described in Woodhall et al. (2008). Inoculum consisted of 5% of the volume of compost by weight. Compost was John Innes No. 3 and plants were grown for two weeks at 20°C in the glasshouse under 16h light and 8 h darkness. Plants were then harvested, washed and five plants were assigned in asymptomatic, slight, moderate and severe disease categories as described in Goulds and Polley (1990). Plants which were not inoculated but grown under the same conditions were used as healthy. DNA was extracted from the bottom 20 mm of each stem. Samples were placed in 2 ml tube containing 2 ml Buffer A (Promega UK, Southampton) with 100 µl 1 mm diameter glass beads. Tubes were then placed on a Precellys®24 (Bertin Technologies) for one minute at full speed. The mixture was processed using a Wizard Magnetic DNA Purification System for Food (Promega) in conjunction with a Kingfisher ML magnetic particle processor (Thermo Scientific UK Ltd).

DNA was extracted from either 50 g samples for artificially spiked soil or 250 g soil from the field. DNA was extracted from soil as described in Woodhall *et al*. (2012) except that homogenisation of 50 g soil was carried out in a 250 ml Nalgene bottle with 3ml of antifoam B, six 25.4 mm stainless steel ball bearings and 100 ml grinding buffer. Soil was collected from UK fields in spring and autumn 2011. Field samples consisted of 25 sub samples taken over a one hectare area in a grid pattern. The sub samples were thoroughly mixed to homogeneity and 250 g was used for DNA extraction. DNA quality of soil samples was verified using a real-time PCR assay for bacteria (Table 2).

Results

Primer design and assay specificity

Primers RC2F and RC2R amplified a 780 bp fragment for isolates RC1 to RC4. The sequence of each was identical. A forward and reverse primer with probe were designed between nucleotides 186 and 260. Primer and probe sequences for the assay are given in Table 2. The assay was tested with all isolates listed in Table 1. A Ct of below 21 was observed with all eight AG-D isolates but no amplification (Ct 40) was observed with DNA from 31 isolates representing other AGs, including 11 other BNR species. In addition, no amplification (Ct 40) was observed when DNA from pure culture of UK isolates of *Oculimacula acuformis, Oculimacula yallundae, Fusarium culmorum, Fusarium poae, Fusarium graminearum, Microdochium nivale* and *Microdochium majus* were tested.

Assay sensitivity

The relationship between Ct and pure DNA concentration is shown in Figure 1. An R^2 value of over 0.99 was observed and the reaction efficiency was determined to be 101%. The limit of detection was determined to be 5 fg although the detection was inconsistent at this level. A strong linear relationship (R^2=0.92) was also observed for sclerotia spiked into soil and pg of DNA detected (Figure 3). The assay combined with a robust soil DNA extraction method was capable of detecting sclerotia weighing 200 µg in 50 g of soil. With wheat DNA (Figure 3), *R. cerealis* was detected in all inoculated material, including asymptomatic material but not in healthy (uninoculated) material. The average amount of pathogen DNA detected correlated with disease severity in inoculated plants (Figure 3).

Detection in UK field soils

Ninety-two soil samples were collected from 20 different counties. Soil samples were taken from fields where *Allium* (onion and leek), oilseed rape, pasture, potato or wheat was recently grown. *R. cerealis* was detected in 48 of the samples (Table 3). The highest incidence of *R. cerealis* was in soil where wheat was most recently grown (72.2% in spring of 78.9% in Autumn) or under pasture in spring (71.4%). In addition to incidence, the highest levels of *R. cerealis* were also detected in soil from wheat in spring (4782 pg DNA/g soil) and pasture (1186.5 pg DNA/g soil). Incidence and mean levels of *R. cerealis* was lowest in soil samples from potato fields (12% incidence and 9 pg/g level). It was possible to sample nine fields, of potatoes following wheat, both in spring and autumn. Six of these fields tested positive for *R. cerealis* in spring (mean level of positives 972 pg

DNA/g soil, range 41 to 4783) but in autumn only one tested positive. The level of DNA of *R. cerealis* in this particular sample reduced from 338 in spring to 9.6 pg DNA/g soil in autumn.

Discussion

In this study we describe a new qPCR assay based on TaqMan chemistry for the detection of the BNR fungus *R. cerealis*, the causal agent of sharp eyespot in cereals. This assay, designed to a unique SCAR sequence, was highly specific for *R. cerealis*. This was shown by testing the assay with DNA against a wide range of *Rhizoctonia* isolates representing most AGs of binucleate and multinucleate *Rhizoctonia*. Previous studies developing real-time PCR assays for *R. cerealis* (Hamada et al., 2012; Guo et al., 2012) have not demonstrated specificity against such a wide range of closely related AGs. Comprehensive testing of specificity against known isolates of related AGs is essential in studies for *Rhizoctonia*. The host range and biology of individual AGs of *Rhizoctonia* can vary substantially and anastomosis grouping is arguably the single greatest advance in understanding the genetic diversity of the species (Cubeta and Vilgalys, 1997).

In addition to specificity, the developed assay was also highly sensitive. We were able to detect 200 µg of sclerotia in 50 g of soil. This is similar to the previous study for the detection of *R. solani* AG3-PT in soil where a single 200 µg sclerotia was detected in a 250 g bulk soil sample. The assay was also able to detect *R. cerealis* in plants prior to symptom development (asymptomatic). Combined with a robust sampling methodology and an accurate risk framework, the assay could be used to screen soils for the presence of the pathogen to inform planting and crop rotation decisions.

The sensitivity and specificity demonstrated with this assay demonstrates the versatility of using unique SCAR fragments for developing real-time PCR assays. In contrast to assays designed to conserved genes such as rDNA ITS and β-tubulin, assays based on unique sequences such as SCAR fragments ensure the assay designed is not constrained to the location of sequence polymorphisms, likely resulting in more optimised primer design and assays with enhanced performance. Here we demonstrate that a SCAR based *R. cerealis* assay had similar sensitivity to a previously designed assay for *R. solani* AG3-PT (Woodhall et al., 2013) designed to ITS sequences which are present in multiple copies in the fungal genome.

Using the assay to test 92 soil samples taken from fields grown under five different crops showed that *R. cerealis* was detected in over half the fields tested. *R. cerealis* was found present in the majority of the wheat and pasture fields tested. This can be expected since cereals are the main host of the pathogen and the role of a previous cereal crop in building up *R. cerealis* inoculum levels in soil has been reported previously (Colbach et al., 1997; Lemańczyk, 2012). The level of *R. cerealis* detected in soil was higher than described in previous studies. Guo et al. (2012) determined the average levels in *R. cerealis* in four wheat fields ranged from 20.3 to 133.0 pg DNA per g of soil, whilst here we found the average level in wheat fields in the UK was higher with levels of 629.6 pg DNA/g soil observed in spring and 135.82 pg DNA/g soil observed in autumn. Soils originating from fields where potato was grown had the lowest levels of *R. cerealis* detected and the lowest incidence of detection. Growing potatoes in the crop rotation may reduce the abundance of the pathogen in soil. In this study, six fields that tested positive for *R. cerealis* in spring were also sampled again, following potatoes, in Autumn. In five of the fields following potatoes no *R. cerealis* was detected in autumn, and in one instance where it was the levels were reduced considerably (by a factor of 33). *R. cerealis* has been shown to be capable of causing infection on potatoes (Hollins et al., 1983) so this reduction in *R. cerealis* abundance may be due to the heavy tillage activities involved in preparing fields for potatoes. Increased cultivation can break up the hyphal networks or *R. cerealis* or force infectious *R. cerealis* propagules deeper underground.

Here we show that *R. cerealis* is relatively widespread in agricultural soils. Surprisingly, Goll et al. (2014) using a soil baiting method did not isolate *R. cerealis* from 282 European soils, including 60 from the UK. This was attributed to the slow growth of *R. cerealis* suggesting that the baiting method was unsuitable for detection of this pathogen. However, Goll et al (2014) did detect a wide range of other AGs in European soils of both BNR and *R. solani*. In a recent study in North America, *R. solani* AG2-1, AG4 and AG5 were all found on wheat (Broders et al., 2014). In England, AG2-1, AG 5 and AG8 were detected in the soil of 96 wheat fields (Brown et al., 2014). *R. solani* AG5 has been previously shown to cause disease in wheat (Woodhall et al., 2012). Further work is required to determine the relative importance of other AGs of *Rhizoctonia* in causing disease and yield loss of wheat and their relative abundance in UK crop rotations.

In this study we developed a specific and sensitive assay for the causal agent of sharp eyespot in wheat and demonstrated its use with plant and soil material. We also confirmed the widespread presence of *R. cerealis* in UK wheat soils. We also showed that growing potatoes in rotation can considerably reduce the level of *R.*

cerealis DNA detected in a field. Further work is required to determine additional *Rhizoctonia* species present in UK wheat soil and their relative importance in causing disease and how crop rotation strategies can affect their abundance.

Acknowledgments

The authors would like to acknowledge Syngenta Global for funding the PhD studentship of Matthew Brown at the University of Nottingham. Eder Somoza Valdeolmillos was supported by the EU Leonardo da Vinci programme.

References

Alaeddini, R. (2012). Forensic implications of PCR inhibition—A review. *Forensic Science International: Genetics, 6,* 297–305.

Boine, B., Renner, A.C., Zellner, M., & Nechwatal, J. (2014). Quantitative methods for assessment of the impact of different crops on the inoculum density of *Rhizoctonia solani* AG2-2IIIB in soil. *European Journal of Plant Pathology*, *140,* 745-756.

Brown, M.B., Woodhall, J.W., Mooney, S.J., & Ray, R.V. (2014). The occurrence and population dynamics of *Rhizoctonia solani* in soil of winter wheat. *Proeceedings of the Dundee Conference*. *Crop Protection in Northern Britain 2014*, Dundee, UK, 25-26 February 2014, 107-112

Budge, G. E., Shaw, M. W., Colyer, A., Pietravalle, S., & Boonham, N. (2009). Molecular tools to investigate *Rhizoctonia solani* distribution in soil. *Plant Pathology, 58*, 1071–1080.

Chen, H.G., Fang, Z., De, H.L., Lin, L., & Wang, Y.Z. (2005). PCR based detection of *Rhizoctonia cerealis*. *Acta Phytophylacica Sinica, 32,* 261-265.

Clarkson, J.D.S. & Cook, R.J. (1983). Effect of sharp eyespot (*Rhizoctonia cerealis*) on yield losses in winter wheat. *Plant Pathology, 32,* 421–428.

Colbach, N., Lucas, P., Cavelier, N. & Cavelier, A. (1997). Influence of cropping system on sharp eyespot in winter wheat. *Crop Protection, 16*, 415–422.

Cubeta, M.A. & Vilgalys, R. (1997). Population Biology of the *Rhizoctonia solani* complex. *Phytopathology, 87,* 480-484.

Goll, M.B., Schade-Schütze1, A., Swart, G., Oostendorp, M., Schott, J.J., Jaser, B. & Felsenstein, F.G. (2014). Survey on the prevalence of *Rhizoctonia* spp. in European soils and determination of the baseline sensitivity towards sedaxane. *Plant Pathology, 63,* 148-154.

Goulds, A. & Polley, R.W. (1990). Assessment of eyespot and other stem base diseases of winter wheat and winter barley. *Mycological Research, 94*, 819-822.

Guo, Y., Li, W., Sun, H., Wang, N., Yu, H. & Chen, H. (2012). Detection of *Rhizoctonia cerealis* in soil using real-time PCR. *Journal of General Plant Pathology, 78,* 247-254.

Hamada, M,S,, Yin, Y., Chen, H., & Ma, Z. (2011). The escalating threat of *Rhizoctonia cerealis*, the causal agent of sharp eyespot in wheat. *Pest Management Science*, *67*, 1411–1419.

Hamada, M.S., Yin, Y.N. & Ma, Z.H. (2012). Detection of *Rhizoctonia cerealis* in wheat tissues by a real-time PCR assay. *Journal of Plant Pathology 94,* 215-217.

Hollins, T.W., Jellis, G.J. & Scott, P.R. (1983). Infection of potato and wheat by isolates of *Rhizoctonia solani* and *R. cerealis. Plant Pathology 32,* 303–310.

Lees, A. K., Cullen, D. W., Sullivan, L., & Nicolson, M. J. (2002). Development of conventional and quantitative real-time PCR assays for the detection and identification of *Rhizoctonia solani* AG3 in potato and soil. *Plant Pathology 51,* 293–302.

Lemańczyk. G., 2012. The role of the preceding crop and weed control in the transmission of *Rhizoctonia cerealis* and *R. solani* to winter cereals. *Journal of Plant Protection Research 52*, 93-105

Lemańczyk. G., Kwaśna, H. (2013). Effects of sharp eyespot (*Rhizoctonia cerealis*) on yield and grain quality of winter wheat. *European Journal of Plant Pathology* 135, 187–200.

Li, W., Sun, H., Deng, Y., Zhang, A. & Chen, H. (2013). The heterogeneity of the DNA ITS sequences and its phylogeny in *Rhizoctonia cerealis*, the cause of sharp eyespot in wheat. *Current Genetics 60*, 1-9.

Nicholson, P. & Parry, D.W. (1996). Development and use of a PCR assay to detect Rhizoctonia cerealis, the cause of sharp eyespot in wheat. *Plant Pathology 45*, 872–873.

Okubara, P.A., Schroeder, K.L. & Paulitz, T.C. (2008) Identification and quantification of *Rhizoctonia solani* and *R. oryzae* using real-time polymerase chain reaction. *Phytopathology*, *98,* 837-847.

Ophel-Keller, K., Mckay, A., Hartley, D., Herdina, & Curran, J. (2008). Development of a routine DNA-based testing service for soilborne diseases in Australia. *Australasian Plant Pathology*, 37, 243–253.

Ray, R.V., Jenkinson, P., & Edwards, S.G. (2004). Effects of fungicides on eyespot, caused predominantly by *Oculimacula acuformis*, and yield of early-drilled winter wheat. *Crop Protection* 23, 1199-1207.

Sharon, M., Kuninaga, S., Hyakumachi, M., Naito, S. & Sneh, B. (2008). Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. *Mycoscience, 49*, 93–114.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, *28,* 2731–2739.

Woodhall, J.W., Lees, A.K., Edwards, S.G., & Jenkinson, P. (2008). Infection of potato by *Rhizoctonia solani*: effect of anastomosis group. *Plant Pathology*, 57, 897–905.

Woodhall, J.W., Laurenson, L. & Peters J.C. (2012). First report of *Rhizoctonia solani* anastomosis group 5 (AG5) in wheat in the UK. *New Disease Reports 26,* 9.

Woodhall, J. W., Webb, K. M., Giltrap, P. M., Adams, I. P., Peters, J. C., Budge, G. E., & Boonham, N. (2012). A new large scale soil DNA extraction procedure and real-time PCR assay for the detection of *Sclerotium cepivorum* in soil. *European Journal of Plant Pathology, 134,* 467–473.

Woodhall, J.W., Adams, I.P., Peters, J.C., Harper, G. & Boonham, N. (2013). A new quantitative real-time PCR assay for *Rhizoctonia solani* AG3-PT and the detection of AGs of *Rhizoctonia solani* associated with potato in soil and tuber samples in Great Britain. *European Journal of Plant Pathology 136,* 273-280.

Yang,S., Lin,S., Kelen,G.D., Quinn,T.C., Dick, J.D., Gaydos, C. A., & Rothman, R. E. (2002). Quantitative multiprobe PCR assay for simultaneous detection and identification to species level of bacterial pathogens. *Journal of Clinical Microbiology, 40,* 3449–3454.

1

2

3

Table 1. Reference list of *Rhizoctonia* isolates used in this study including isolate codes, source, anastomosis group, original host and origin

Isolate code	Species group	Other codes	AG	Original host	Origin
cc1996	*R. solani*		2-1	Cauliflower	UK
cc2314	*R. solani*		2-1	Broccoli	UK
J11	*R. solani*		2-2IIIB	Potato	USA
1847	*R. solani*	76132;IPO BIR01	2-BI	Soil	Japan
Rs08	*R. solani*		3-PT	Potato	UK
Rs10a	*R. solani*		3-PT	Potato	UK
cc1938	*R. solani*		4 HG-II	Iris	The Netherlands
J15	*R. solani*		4 HG-II	Potato	USA
44RS	*R. solani*	ATCC 14007	4 HG-III	Sugar beet	USA
Rs09A	*R. solani*		5	Potato	UK
T1	*R. solani*		5	Couch grass	UK
cc1891	*R. solani*	06-01, OMT-1-1	6 HG-I	Not known	Not known
cc1911	*R. solani*	R28, SH51	8	Barley	UK
cc1843	*R. solani*	CBS 970.96, ATCC 90334	9	Potato	USA
1844	*R. solani*	CBS 972.96	10	Clover	Australia
1846	*R. solani*	CBS 974.96, ATCC 90857	11	*Lupinus angustifolius*	Australia
137	BNR	C66s	A	Soil	Japan
90	BNR	UCD	Ba	Rice	USA
55	BNR	C-455	Bb	Rice	Japan
54	BNR	SRZ	B(o)	Sweet Potato	Japan
RC1	BNR		D	Wheat	UK
RC2	BNR		D	Wheat	UK
RC3	BNR		D	Wheat	UK
RC4	BNR		D	Wheat	UK
RC5	BNR		D	Wheat	UK
RC6	BNR		D	Wheat	UK
RC7	BNR		D	Wheat	UK
7	BNR	BN1	D (CAG1)	*Agrostis* sp.	USA
1921	BNR		E	Soil	UK
9	BNR	BN3	E (CAG3)	Peanut	USA
10	BNR	BN38	F (CAG4)	Soybean	USA
41	BNR	STC9	H	Soil	Japan
1923	BNR		I	Soil	UK
1922	BNR		K	Soil	UK
155	BNR	AC02-26	L	Not known	Not known
134	BNR	580-111-DTR	R (CAG5)	Soil	Canada
18	BNR	BN22	S (CAG7)	*Pittospoirum* sp.	USA
cc43	BNR		unknown	Potato	UK
cc1987	*R. zeae*		WAG-Z	Soil	UK

1 Table 2. Primers and probes used in the study

Target	Primer/probe name	Sequence	Source
Rhizoctonia cerealis PCR	Rc2F	AAAACTGGCAACCCTTGGTG	Nicholson and Parry, 1996
	Rc2R	TAACTCACCACTCCAGCCGTT	
Rhizoctonia cerealis TaqMan	RcF	AAAGCATCGTCGCCATGAG	This study
	RcR	CTGCCAACACACCGACATGT	
	RcP*	ATAAAATGGAAGGTAGGTGCGGGTG CATAG	
Universal bacteria TaqMan	P891F	TGGAGCATGTGGTTTAATTCGA	Yang et al., 2002
	P1033R	TGCGGGACTTAACCCAACA	
	UniProbe*	CACGAGCTGACGACARCCATGCA	

2 *probes were labelled with FAM-TAMRA
3
4
5 Table 3. Amount of *Rhizoctonia cerealis* DNA detected (pg/g soil) in 92 UK field soils under different crops at
6 two time points (spring and autumn)

Previous crop	Number of fields sampled	Number of fields *Rhizoctonia cerealis* detected	% Incidence *Rhizoctonia cerealis* detection	Mean DNA quantity of *Rhizoctonia cerealis* (pg DNA/ g soil) detected in positive samples	Range (pg DNA/g soil)
Spring					
Allium	11	6	54.5	53.2	14.7 to 130.2
Oilseed Rape	2	0	0	-	-
Pasture	7	5	71.4	1186.5	67.4 to 3965.8
Potato	4	0	0	-	-
Wheat	18	13	72.2	629.6	38.4 to 4782.9
Autumn					
Allium	2	0	0	-	-
Oilseed Rape	14	7	50.0	178.7	21.9 to 510.8
Potato	15	2	13.3	79.9	9.6 to 150.2
Wheat	19	15	78.9	135.82	12 to 446.0

7
8

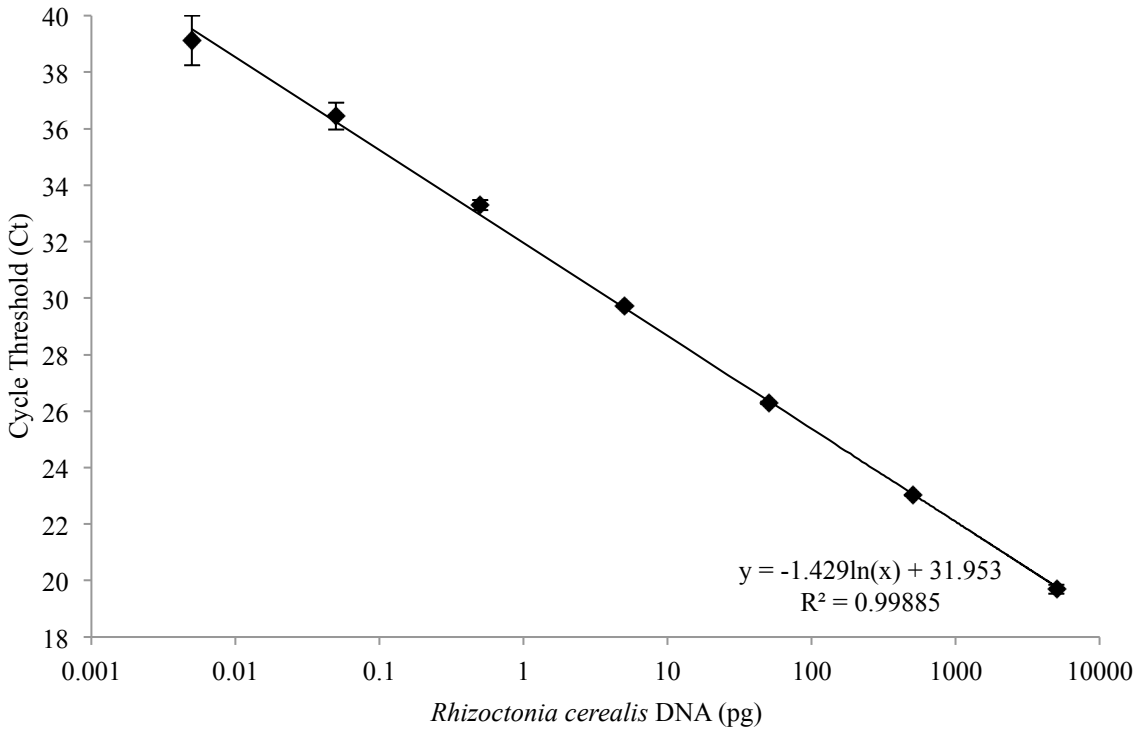

Figure 1. Real-time PCR cycle threshold with *Rhizoctonia cerealis* DNA of known concentration from pure culture. Error bars represent the standard error of the mean.

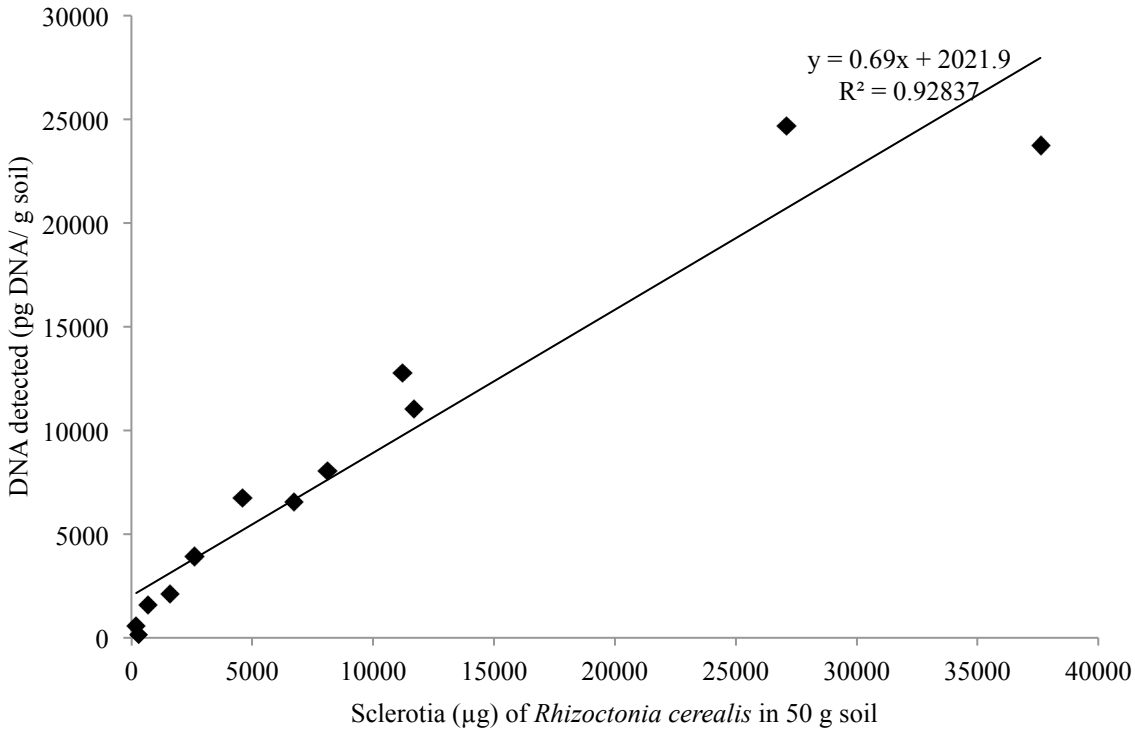

Figure 2. Relationship between detected DNA (pg/g soil) and weight of *Rhizoctonia cerealis* sclerotia spiked into 50 g soil samples.

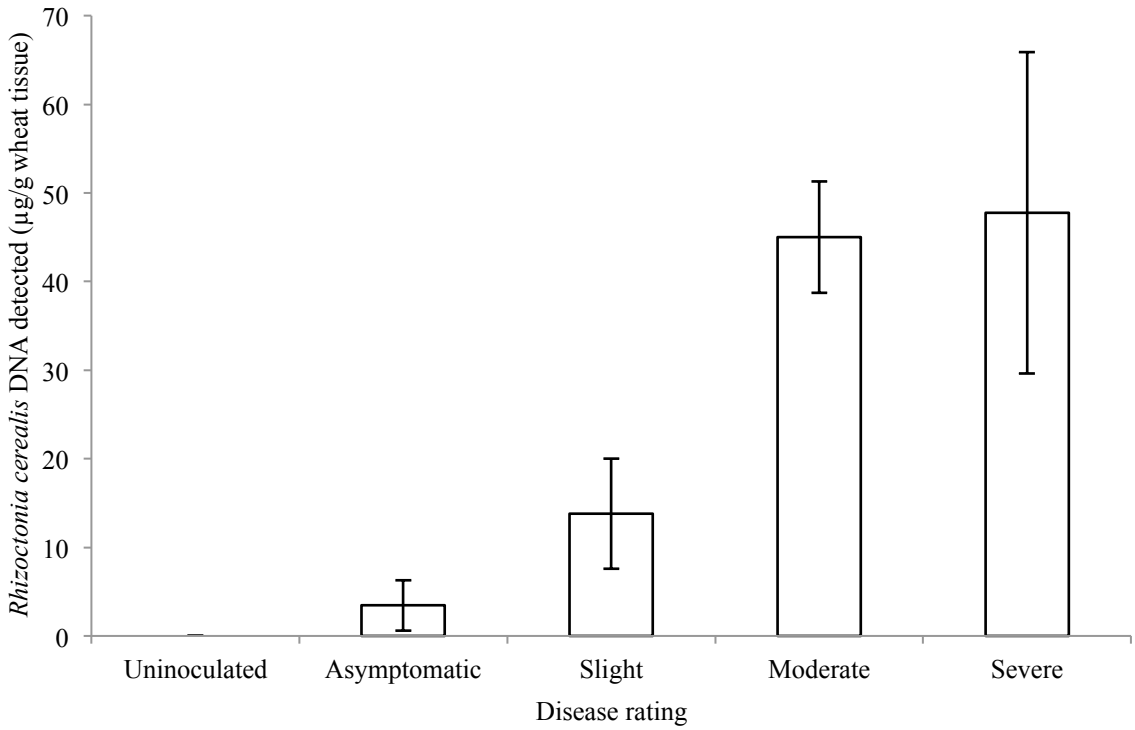

Figure 3. Amount of DNA detected in asymptomatic wheat stems and stems with slight, moderate and severe symptoms. Error bars represent the standard error of the mean.