A bioassay to compare the disease suppressive capacity of pasture soils

B.E.A. Dignam[1,2], M. O'Callaghan[1,2], L.M. Condron[1], J.M. Raaijmakers[3], G.A. Kowalchuk[4] and S.A. Wakelin[1,2]

[1]Bio-Protection Research Centre, PO Box 85084, Lincoln University 7647, Canterbury, New Zealand
[2]AgResearch Ltd, Lincoln, Private Bag 4749, Canterbury 8140, New Zealand
[3]The Netherlands Institute of Ecology, The Netherlands
[4]Institute of Environmental Biology, Utrecht University, The Netherlands
Corresponding author: bryony.dignam@agresearch.co.nz

Abstract Dynamic pathogen complexes can develop under pastures, thereby substantially reducing potential productivity. Suppression of such pathogen complexes is therefore of great importance, and bioassays can quantify disease suppression in soils. This study describes the development of a pasture-relevant system: *Rhizoctonia solani* AG 2-1 induced damping-off (wirestem) of kale (*Brassica oleracea)*. As kale is not a component of traditional ryegrass clover pasture swards, the assay allows assessment of general disease suppression, considered more enduring in multiple-host-multiple-pathogen systems. A pathogenic *Rhizoctonia solani* isolate was obtained from New Zealand pastoral soil. Inoculation of soils with this isolate resulted in a level of damping-off disease comparable to that induced by reference *Rhizoctonia solani* isolate Rs043-2. Significantly different levels of inoculum-induced disease incidence and progression were found in four distinct pastoral soils. In combination with soil physicochemical data and environmental DNA approaches, this bioassay can be used to further advance understanding of the influence of farm management practices on disease suppression in pasture soils.

Keywords soil-borne plant disease, pastures, disease suppression.

INTRODUCTION

Diverse and dynamic pathogen complexes can develop under pastoral soils (Skipp & Watson 1996). The diseases they cause constrain the potential productivity of pastoral agriculture and reduce water and nutrient use efficiency (Baligar et al. 2001). A range of soil-borne pathogens, including fungi, oomycete and nematodes, causes diseases in New Zealand pastures (Falloon 1985; Skipp & Christensen 1989; Waipara et al. 1996; Sarathchandra et al. 2000; Harvey & Harvey 2009). Because the disease symptoms are often only directly observable below-ground, the prevalence of these diseases is largely unknown and losses attributed to soil-borne diseases are often underestimated and notoriously difficult to quantify. However, the consensus of studies from New Zealand suggests that production losses are likely to be between 40-50% (Skipp & Watson 1996 and references therein).

Control of soil-borne diseases in pastoral systems is inherently difficult. Approaches must account for multiple-host-multiple-pathogen

interactions, in addition to other system-specific issues. Additional difficulties stem from limited opportunities to use break-crops, pathogen complexes that increase in inoculum potential over time, and the expansive nature of pastures that reduces the economic and practical viability of delivering, for example, chemical controls.

Disease suppressive soils naturally reduce the severity of disease caused by soil-borne phytopathogens (Weller et al. 2002). There are two distinct types of disease suppression: general suppression is attributed to the competitive activity of the total microbiota, which competes with the pathogenic microbiota for resources present in the soil (Weller et al. 2002), and specific suppression is attributed to activities of an individual (or specific group of) microorganism antagonistic towards the pathogen or pathogen complex (Weller et al. 2002). General disease suppression is potentially higher in pasture soils than arable soils due to the increased microbial diversity supported by diverse above-ground vegetation (Garbeva et al. 2006). Thus, managing soil ecosystems towards increased disease suppression represents a sustainable approach for the control of soil-borne diseases in pastures.

Well-characterised examples of specific disease suppression in arable systems, such as soils suppressive to take-all disease of wheat (Weller et al. 2002) or black root rot of tobacco (Almario et al. 2014), have provided a wealth of information regarding the microbial taxa and functions underlying this phenomenon (Raaijmakers et al. 2009). However, comparatively little is known about how management practices influence general disease suppression in pasture systems. To identify opportunities to manage this biological phenomenon, it is important to first characterise the abiotic and biotic parameters influencing the progression of plant disease in soils.

Plant-pathogen bioassays can be used to quantify disease suppression in soils by comparison of disease symptoms in inoculated treatments, such as *Rhizoctonia solani*-induced damping-off disease of sugar beet (Mendes et al. 2011). Furthermore, Skipp & Sarathchandra (1999) demonstrated that such assays (white clover seedling emergence in soil inoculated with *Cylindrocladium scoparium* Morgan) could be used to quantify the deleterious effect of soil-borne phytopathogens in pasture systems, thereby providing a comparative measure of disease suppression that is useful for monitoring the influence of management practices on soil disease suppressive capacity in farming systems.

In this study, a pasture-relevant plant-pathogen bioassay was developed and tested. The assay follows the general strategy adopted by Mendes et al. (2011). As the aim was to quantify the general disease suppressive capacity of pastoral soils, kale (*Brassica oleracea*) was used as a plant species unrelated to ryegrass and white clover, and a ubiquitous, broad host range fungal pathogen (*Rhizoctonia solani*) was selected as the pathogen to be inoculated into soil.

MATERIALS AND METHODS

Rhizoctonia solani isolates

Two *Rhizoctonia solani* (*R. solani*) isolates were used in the development of the disease assay. Rs043-2, obtained from a culture collection held at the New Zealand Institute for Plant & Food Research, had been isolated from potato tubers with sclerotia (black scurf) and characterised as belonging to the anastomosis group (AG) 2-1 (Das et al. 2014). Rs37 was isolated in this study from wirestem lesions (damping-off) of kale plants grown in pastoral soil (see below).

Isolation of putative *Rhizoctonia solani*

In preliminary pathogenicity testing of isolate Rs043-2 in pasture soil (Kurow; Table 1), wirestem lesions developed on stem bases of kale seedlings planted in uninoculated soil. Disease symptoms were comparable to those induced by Rs043-2 in the inoculated treatment.

To isolate the causative agent, diseased stem bases were immersed in 0.2% NaOCl for 1 min, rinsed with sterile water and plated directly onto 1.5% water agar. Plates were stored at 20°C in the dark and visually assessed for Rhizoctonia-like fungi (RLF) after 24 and 48 h. After 48 h, RLF were transferred to half strength potato dextrose agar (PDA) amended with streptomycin sulphate (200 mg/litre). Isolates were maintained on non-amended, one-fifth strength PDA.

Identification and characterisation of *Rhizoctonia solani*

To confirm the identity of RLF isolates, colony morphology was observed by hyphal staining with Lactophenol blue solution (Merck) and light microscopy. To determine AGs of *R. solani* isolates, sequence homology of the internal transcribed spacer regions (ITS rRNA gene) was used. Isolates with morphological characteristics similar to *R. solani* (reference strain Rs043-2) were grown on 1.5% water agar amended with streptomycin sulphate (200 mg/ml) for 48 h at 25°C in the dark. Mycelia were collected directly from the plates into 1.5 ml tubes and frozen with liquid nitrogen. Frozen mycelia were ground with sterilised micro-pestles and genomic DNA extracted using Qiagen DNeasy Plant Mini Kit according to the manufacturer's instructions.

PCR amplification of the ITS1-5.8S-ITS2 region used primers ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') and ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') (White et al. 1990; Integrated DNA Technologies). Each 25 µl reaction mixture contained 200 nM of each primer, 5 mM dNTPs, 15 mM $MgCl_2$, 1 U MyHSTaq™ DNA Polymerase (BioLine) and 4 µl of template DNA. Thermocycling conditions consisted of an initial denaturation at 95°C for 3 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The presence of amplicons of the expected size was validated by agarose gel electrophoresis.

Purified PCR products were sequenced in both forward and reverse (Lincoln University, NZ), and ITS rRNA consensus sequences were generated for each isolate using Geneious version 7.0.5 (http://www.geneious.com; Kearse et al. 2012). Nucleotide BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi; Altschul et al. 1990) aligned the consensus sequences with publicly available sequences already assigned to *R. solani* AGs.

Soil sampling

Soil was collected from four locations identified as representative of the three major soil orders used for pastoral production in New Zealand. Soil orders were classified according to Hewitt (1998) as Brown, Recent and Pumice. Brown soils were collected from two locations: Lincoln (Lin) and Eyrewell (Eyr). Forty-three percent of New Zealand's soil is classified as Brown (Ministry for the Environment, 2007). A Recent soil was collected from Kurow (Kur) and a Pumice soil from Rotorua (Rot). Soils were sampled from a single point at each location to the depth of the plant roots present. Soil was sieved to 2 mm to remove stones and plant debris, and stored at 4°C prior to use. Soil physical-chemical analyses were performed by Hills Laboratories (Christchurch/Hamilton).

Plant-pathogen bioassay and disease assessment

Sixteen kale seeds (cv. Caledonian) were sown into 7 × 7 × 8 cm pots (with sealed bases) containing 170 g of field-moist soil, and seeds were covered with a further 20 g of field moist soil. Pots were adjusted to 70% of the soil's maximum water holding capacity (MWHC) by watering to weight every other day. Experiments were conducted in a growth room with 16 h light at 22°C and 8 h dark at 15°C. Seedlings were thinned to 10 per pot 5 days after sowing and the soil inoculated with *R. solani* 7 days after sowing. Two mycelial plugs (6 mm in diameter) of a 7-day-old one-fifth PDA *R. solani* culture were buried approximately 1 cm under the soil surface in opposite corners of each pot. Sterile one-fifth PDA plugs were added to the uninoculated, control treatment.

Damping-off disease in cruciferous brassica spp. is characterised by the development of wirestem lesions (Carling & Sumner 1992). Lesions develop where the base of the plant stem contacts the soil surface; they are initially dark brown-grey in colour and become sunken so that the stem resembles a wire (Keinath & Farnham 1997). Wirestem lesions were recorded as present when 1 to 3 mm of the stem base was dark brown-grey in colour. By scoring each individual plant for the presence of wirestem lesions, disease progress was measured as the percentage of plants, per pot, with damping-off disease at 2-day intervals up to 20 days post inoculation. Following disease assessment, the fungal pathogen was re-isolated from the lesions of five plants per treatment as described above.

Experimental design

To establish the pathogenic potential of *R. solani* isolate Rs37 in both Kur and Lin soils, the initial experiment compared damping-off disease progress on kale plants inoculated with Rs37 and the positive control isolate Rs043-2. To establish whether the level of induced disease was comparable across New Zealand pasture soils, a subsequent experiment assessed damping-off disease progress on kale plants in Kur, Lin, Eyr and Rot soils inoculated with Rs37. Pots were inoculated with mycelial (inoculum) and sterile (control) agar plugs as described above. Split-plot, randomised experimental designs were used, with six or eight replicates per treatment.

Statistical analysis

For comparison of disease progress data, the area under the disease progress curve (AUDPC) per pot was calculated from the percentage of plants with damping-off disease over the 20-day period. In addition, disease incidence (DI) was calculated as the percentage of diseased plants at 20 days post inoculation.

Analyses of variance were performed in Genstat for Windows (16th Edition SP1) to test for significant effects of *R. solani* isolate or soil on AUDPC and DI. Following ANOVA, Tukey's method of pairwise comparisons was used to test for significant differences between treatment means. Block structures were applied to all analyses of disease progress data and differences were considered significant when $P \leq 0.05$.

RESULTS

Identification of RLF isolate

BLASTn analysis of the nuclear ITS rRNA sequence of *R. solani* isolate Rs37 revealed that it was identical in nucleotide composition to isolates assigned to AG 2-1. Rs37 had 100% sequence identity with GenBank accession KF870933, a strain characterised as AG 2-1 by anastomosis fusion with AG 2-1 tester isolates (Broders et al. 2014). Furthermore, subsequent closest matches in alignments were with accessions identified as other AG 2-1 isolates.

Soil properties

Soil physical and chemical characteristics are given in Table 1. The soils selected varied in bulk density, pH, organic matter content (anaerobically mineralisable N) and fertility status (Table 1), and thus represent a wide range of New Zealand pasture soils.

Pathogenicity testing

In both Kur and Lin soils, *R. solani* isolate Rs37 was capable of causing a similar level of disease on kale as compared to the reference isolate Rs043-2 (Figure 1 and Figure 2). Disease progression, AUDPC ($P=0.002$) and DI ($P<0.001$), was significantly greater in *R. solani* inoculated treatments than the uninoculated control treatments. Differences in mean AUDPC and DI were not statistically significant between treatments inoculated with Rs37 and Rs043-2 in both Kur (Rs37 DI =78%; Rs043-2 DI = 95%) and Lin (Rs37 DI = 68%; Rs043-2 DI = 80%)

Table 1 Selected physical and chemical characteristics determined for the four soils[1].

		Lincoln	Eyrewell	Rotorua	Kurow
NZ Soil Order		Brown	Brown	Pumice	Recent
pH	pH Units	5.6	6.1	5.1	6.6
Total Carbon	%	2.5	4.7	6.8	2.4
Total Nitrogen	%	0.25	0.38	0.64	0.26
C/N	Ratio	10	12.3	10.7	9.3
Anaerobically Mineralisable N	µg/g	29	78	115	69
Available Nitrogen	kg/ha	45	110	103	92
Total Phosphorus	mg/kg	698	725	1,404	670
Olsen Phosphorus	mg/litre	29	43	48	18
Sulphate Sulphur	mg/kg	15	17	14	6
Volume Weight (bulk density)	g/ml	1.02	0.94	0.6	0.88

[1]An expanded list of soil properties for these soils is available on request.

soils. While kale seedlings remained free of disease symptoms in the Lin soil uninoculated treatment, damping-off symptoms did develop on seedlings grown in uninoculated Kur soil (DI = 38%). *Rhizoctonia solani* was re-isolated from the wirestem lesions of both inoculated treatments and the uninoculated Kur soil treatment.

Disease progression in New Zealand pasture soils

Optimisation of the plant-pathogen bioassay determined that mycelial agar plugs 6 mm in diameter provided an optimal inoculum level for comparison of disease progress among soil types (data not shown). There were significant differences in disease progression in the four soils (AUDPC and DI) between *R. solani* Rs37-inoculated treatments (Figure 3). The statistical separation of inoculated treatments based on disease progression differed between DI and AUDPC measurements. DI was highest in Rot soil (88%) and significantly higher in this treatment than in Eyr soil (59%) (Figure 3a). In contrast, AUDPC was highest for Kur soil (548) and significantly higher for this treatment than for Lin (342) or Eyr (321) soils (Figure 3b). There was evidence of background disease pressure in two of the four soils tested; damping-off disease symptoms developed on seedlings grown in uninoculated Eyr (DI = 1%) and Kur (DI = 16%) soils. *Rhizoctonia solani* was re-isolated from the wirestem lesions of all inoculated treatments and the Kur soil uninoculated treatment, but not the Eyr uninoculated treatment.

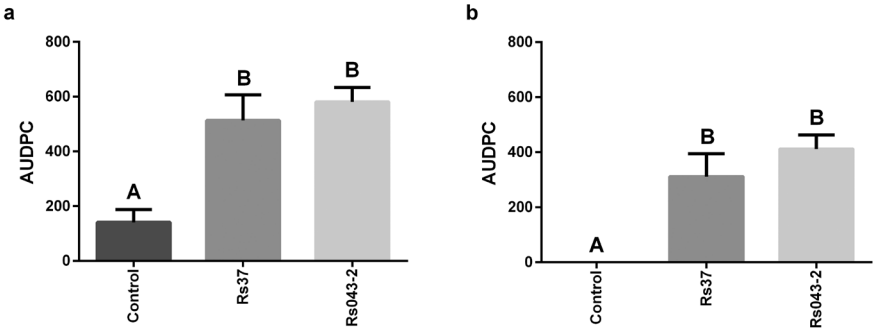

Figure 1 Progress (AUDPC) of *Rhizoctonia solani* induced damping-off (wirestem) disease on kale seedlings grown in (a) Kurow soil or (b) Lincoln soil, and inoculated with isolates Rs37 and Rs043-2 (mean ± SEM, N=6). Letters above the bars indicate significantly different treatments (P<0.05, Tukey).

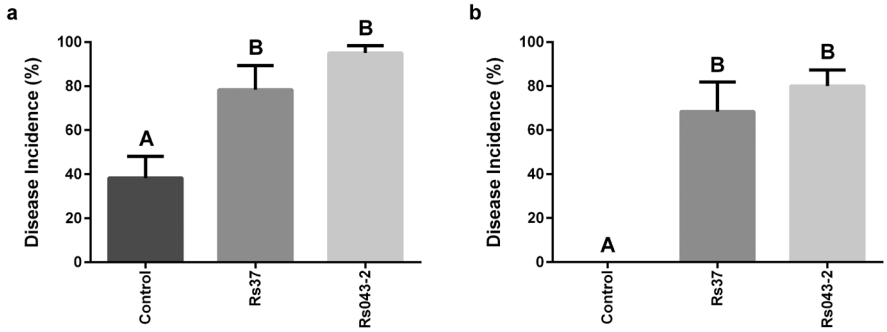

Figure 2 *Rhizoctonia solani* induced damping-off (wirestem) disease incidence (DI) on kale seedlings grown in (a) Kurow soil or (b) Lincoln soil, and inoculated with isolates Rs37 and Rs043-2 (mean ± SEM, N=6). Letters above the bars indicate significantly different treatments (P<0.05, Tukey).

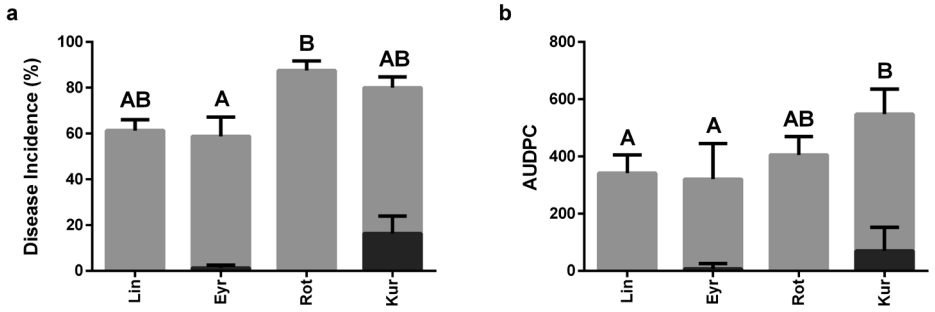

Figure 3 (a) Damping-off disease progress (DI) and (b) AUDPC, of *Rhizoctonia solani* Rs37 on kale seedlings (mean ± SEM, N=8), compared between four New Zealand pasture soils: Kurow (Kur), Lincoln (Lin), Eyrewell (Eyr) and Rotorua (Rot). For soils in which damping-off disease symptoms developed in the uninoculated treatment, disease progress (dark grey) is represented as a proportion of disease progress in the inoculated treatment (light grey). Letters above the bars indicate significantly different inoculated treatments ($P<0.05$, Tukey).

DISCUSSION

The increasing value of crop production in New Zealand pastures places emphasis on the need for practical and sustainable disease control options that enhance plant health and increase water and nutrient (e.g. fertiliser) use efficiency. This study has developed a rapid and robust plant-pathogen bioassay that provides a comparative measure of *R. solani*-induced damping-off disease incidence and progression on kale plants. The assay has proven sufficiently sensitive to distinguish between levels of inoculum-induced disease across a range of New Zealand pastoral soils.

This assay will be of particular value for monitoring the spatial and temporal variability of disease progression in the soils of various pasture systems. It could therefore be implemented to assess the success of disease mitigation strategies over time and also to identify potential management opportunities that exploit the disease suppressive capacity of soils to reduce disease pressure. Biotic and abiotic soil parameters shown to correlate with the suppression of inoculum-induced disease should present new opportunities to manage the disease suppressive components of pasture soils. Furthermore, the assay may be applied for the investigation of specific disease suppression in cropping systems where brassicas form a frequent component of the crop rotation. Application of the assay to a greater range of soils is required to further validate its efficacy among pastoral and agricultural soil systems.

Rhizoctonia solani strains are ubiquitous, broad host range, soil-borne plant pathogens (Ogoshi 1987) considered to be the cause of the most common diseases of brassica seedlings (Stewart & Charlton 2006). A pathogenic *R. solani* isolate, Rs37, was obtained from a New Zealand pasture soil. Analysis of its ITS rRNA gene sequence characterised Rs37 as belonging to AG 2-1, an AG group shown to cause damping-off of *brassica* spp. globally (Lamprecht et al. 2011). The strain is available on request from AgResearch (contact authors) and will be available from the International Collection of Microorganisms from Plants (ICMP) in due course. Isolate Rs37 expressed a similar pathogenic potential to the reference strain Rs043-2, an AG 2-1 isolate previously shown to be an effective source of inoculum in a potato-based disease assay (Bienkowski et al. 2010). Inoculation of four pastoral soils with Rs37 resulted in a level of damping-off disease that was suitable for comparison of disease incidence and progress between soil types. Due to the broad host range of the pathogen used, the bioassay should prove suitable for application across diverse agricultural systems.

For the assessment of disease suppression in pastures, kale was used as the host plant, rather than a grass or a legume. It is proposed that measuring disease incidence on host plants unrelated to a system's current crop species will provide a better measure of underlying general, rather than specific, disease suppression. To validate this approach, the capacity of a soil to suppress multiple soil-borne pathogens will need to be quantified using multiple bioassays incorporating host plants both related and unrelated to the plant species present in the pastoral system. Specific disease suppression can be induced by repeated monoculture cultivation (Weller et al. 2002), and therefore, enhanced resistance to host-specific pathogens may develop over the life of a pasture. However, broad host range pathogens, such as *Pythium*, *Rhizoctonia* and *Fusarium* spp., will not necessarily be suppressed in long lived, multiple-host-multiple-pathogen pasture systems and are therefore ideal candidates for the development of such assays.

Although brassica species, such as kale, are not a component of traditional pasture swards, they are widely used as animal forage crops in New Zealand pasture systems. Inconsistencies in localised and seasonal establishment of brassica species, which can affect their reliability as crops, have been attributed to insect pests and fungal pathogens (Stewart & Charlton 2006). The bioassay developed can also be used to detect the presence of such pathogens by monitoring disease incidence in uninoculated soil.

It was interesting to note that background disease pressure was detected in two of the four soils tested. Previous studies of disease suppression in New Zealand pasture soils have inoculated pre-sterilised soil as a control treatment and to determine the pathogenic potential of fungal isolates in the absence of potential interactions with naturally occurring background pathogens (for example, Skipp & Sarathchandra 1999). In contrast, uninoculated, non-sterile soil was used as the control treatment in this study. This has provided insight into the prevalence of related diseases in the pastoral systems tested.

Rhizoctonia solani was isolated from the wirestem lesions of plants in uninoculated Kur soil. In the presence of a suitable plant host, *R. solani* field populations can increase over time (Ogoshi 1987). As such broad host range pathogens have the potential to carry over through break crops, their presence in pasture systems also has implications for the productivity of subsequent agricultural crops planted in such fields.

The statistical separation of inoculated soils differed between DI and AUDPC disease progression measurements. This difference is attributed to a sharp rise in the mean number of diseased plants in Rot soil from 16 to 18 days post inoculation (data not shown). Whilst this increase meant that, at the end of the assay, DI was highest in the Rot soil, AUDPC of the Rot soil was not increased to above that of the Kur soil. DI provides a finite measure of disease in each soil, only accounting for the mean number of plants expressing disease symptoms at the end of the assay. The AUDPC provides a cumulative measure of disease progression and, given that disease symptoms typically worsen over the course of the assay (anecdotal evidence), provides an indirect measure of disease severity. In the use of this assay, it is recommended that consideration be given to the AUDPC in combination with the plot of the disease progress curve, generated by sequential measurements of disease incidence over the course of the assay. The AUDPC provides a comparative measure of general disease suppression that will identify soils in which disease severity is reduced relative to other soils tested. Furthermore, plotting the disease progress curve will allow any substantial changes in disease incidence at a given time point to be easily visualised.

Coupled with molecular analyses of environmental DNA and detailed soil physicochemical data, this bioassay can be used to investigate the relationship between the presence and abundance of putative disease suppressive microbial taxa, abiotic soil parameters and disease progression. Thus, it provides the foundation necessary for future research aiming to understand how farm management practices influence disease suppression in pasture soils.

ACKNOWLEDGEMENTS

We acknowledge Dr Farhat S.A. Shah (Plant & Food Research) and Dr Damian Bienkowski (Bio-Protection Research Centre), who supplied *R. solani* isolate Rs043-2, and Dr Alasdair Noble (AgResearch, Lincoln) for statistical advice. We thank the anonymous reviewer for their expert comment and suggestions on a draft of this manuscript. This research was funded by a Bio-Protection Research Centre PhD scholarship.

REFERENCES

Almario J, Moënne-Loccoz Y, Muller D 2013. Monitoring of the relation between 2,4-diacetylphloroglucinol-producing *Pseudomonas* and *Thielaviopsis basicola* populations by real-time PCR in tobacco black root-rot suppressive and conducive soils. Soil Biology and Biochemistry 57: 144-155.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ 1990. Basic local alignment search tool. Journal of Molecular Biology 215(3): 403-410.

Baligar VC, Fageria NK, He ZL 2001. Nutrient use efficiency in plants. Communications in Soil Science and Plant Analysis 32(7-8): 921-250.

Bienkowski D, Stewart A, Falloon R, Braithwaite M, Loguercio L, Hicks E 2010. A disease assay for *Rhizoctonia solani* on potato (*Solanum tuberosum*). New Zealand Plant Protection 63: 133-137.

Broders K, Parker ML, Melzer M, Boland GJ 2014. Phylogenetic diversity of *Rhizoctonia solani* associated with canola and wheat in Alberta, Manitoba, and Saskatchewan. Plant Disease 98(12): 1695-1701.

Carling DE, Sumner DR 1992. *Rhizoctonia*. In: Singleton LL, Mihail JD, Rush CM ed. Methods for research on soilborne phytopathogenic fungi. American Phytopathological Society, Minnesota, USA. Pp. 157-165.

Das S, Shah F, Butler R, Falloon R, Stewart A, Raikar S, Pitman A 2014. Genetic variability and pathogenicity of *Rhizoctonia solani* associated with black scurf of potato in New Zealand. Plant Pathology 63(3): 651-666.

Falloon RE 1985. Fungi pathogenic to ryegrass seedlings. Plant and Soil 86(1): 79-86.

Garbeva P, Postma J, Van Veen J, Van Elsas J 2006. Effect of above-ground plant species on soil microbial community structure and its impact on suppression of *Rhizoctonia solani* AG3. Environmental Microbiology 8(2): 233-246.

Harvey IC, Harvey BM 2009. Pasture diseases in New Zealand. Bio-Protection Research Centre, Lincoln, New Zealand. 144 pp.

Hewitt AE 1998. New Zealand soil classification. Landcare Research Science Series No.1, 2nd edition. Manaaki Whenua Press, Lincoln, New Zealand. 133 pp.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647-1649.

Keinath AP, Farnham MW 1997. Differential cultivars and criteria for evaluating resistance to *Rhizoctonia solani* in seedling *Brassica oleracea*. Plant Disease 81(8): 946-952.

Lamprecht SC, Tewoldemedhin YT, Calitz FJ, Mazzola M 2011. Evaluation of strategies for the control of canola and lupin seedling diseases caused by *Rhizoctonia* anastomosis groups. European Journal of Plant Pathology 130(3): 427-439.

Ministry for the Environment 2007. Environment New Zealand 2007. Ministry for the Environment, Wellington, New Zealand. Pp. 217-223.

Mendes R, Kruijt M, De Bruijn I, Dekkers E, Van Der Voort M, Schneider JHM, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, Raaijmakers JM 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332(6033): 1097-1100.

Ogoshi A 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. Annual Review of Phytopathology 25(1): 125-143.

Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y 2009. The rhizosphere: A playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant and Soil 321(1-2): 341-361.

Sarathchandra SU, Watson RN, Skipp RA, Burch G, Brown JA, Cox NR 2000. Microbial pathogens and plant parasitic nematodes in pastures with declining vigour. New Zealand Journal of Agricultural Research 43: 549-558.

Skipp RA, Christensen MJ 1989. Fungi invading roots of perennial ryegrass (*Lolium perenne L.*) in pasture. New Zealand Journal of Agricultural Research 32: 423-431.

Skipp RA, Watson RN 1996. Disease complexes in New Zealand pastures. In: Chakraborty S, Leath KT, Skipp RA, Pederson GA, Bray RA, Latch GCM, Nutter FW ed. Pasture and forage crop pathology: proceedings of a trilateral workshop held at the Mississippi State University, Mississippi, 10-13 April 1995. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Wisconsin, USA. Pp. 429-451.

Skipp RA, Sarathchandra S 1999. Bioindicator tests of pathogen potential and disease suppressiveness of pasture soil. In: Magarey RC ed. Proceedings of the First Australasian Soil-Borne Disease Symposium. BSES, Brisbane, Australia. Pp. 146-147.

Stewart AV, Charlton JFL 2006. Pasture and forage plants for New Zealand. Grassland research and practice series No 8, 3rd edition. New Zealand Grassland Association, Dunedin, New Zealand. 128 pp.

Waipara NW, Di Menna ME, Cole ALJ, Skipp RA 1996. Potential pathogenicity of pasture plant root-colonising fungi to seedlings of legumes and grasses. Proceedings of the 49th New Zealand Plant Protection Conference: 212-215.

Weller DM, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annual Review of Phytopathology 40: 309-348.

White TJ, Bruns T, Lee S, Taylor J 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ ed. PCR protocols: a guide to methods and applications. Academic Press, New York, USA. Pp. 315-322.