Potential risk of contaminated seed as a source for foliar disease in barley – should we take the risk more seriously?

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Introduction

Barley is an important crop in Scotland, where annual production comprises 0.38 million tonnes of winter barley and 1.46 million tonnes of spring barley, and has a value at the farm gate of £415m. Two major fungal pathogens of barley are rhynchosporium (*Rhynchosporium secalis*) and ramularia (*Ramularia collo-cygni*). Barley is continually under threat from new pathogens and races of existing pathogens, and the development of new molecular diagnostics for both pathogens (Fountaine *et al.*, 2007; Havis *et al.*, 2006) has increased our ability to study these major pathogens and understand the importance of seed-borne infections.

Importance of seed-borne infection of *Ramularia collo-cygni*

Ramularia leaf spot caused by *Ramularia collo-cygni* is a relatively new foliar disease in Scotland. By using a molecular diagnostic test specific to the fungus (Havis *et al.*, 2006), it has been possible to show that the pathogen was commonly found on barley seed. Seed-borne infection is now considered to be a major source of the pathogen (Havis & Oxley, 2008). *Ramularia collo-cygni* develops asymptomatically within the plant, colonising new leaves as they develop. Visible symptoms appear on the leaves, stems and awns after the plant is exposed to weather or physiological stresses. Although airborne spores are another source of infection for barley plants, analysis of spore traps situated in Scotland suggests that most airborne spores are detected late in the season once symptoms are widespread. Airborne spores may therefore have greater importance in infecting seed for the disease epidemic in the following year.

Ramularia leaf spot is now becoming more widely recognised throughout the UK. A study of seed samples harvested from different regions in the UK showed seed stocks taken from Scotland, Lincolnshire, Cambridgeshire and Somerset in 2005 and 2006 to be contaminated with *R. collo-cygni* (Havis & Oxley, 2007). The spread of ramularia throughout the UK may either be due to better recognition of symptoms, or possibly as a consequence of movement of contaminated seed from high disease pressure to low disease regions.

Importance of seed-borne infection of *Rhynchosporium secalis*

The potential for *R. secalis* on seed as a major source was reported by Lee *et al.* (2001). Research at Rothamsted confirmed the importance of seed-borne infection and the ability for seed contamination to lead to a symptomless phase of *R. secalis* (Fountaine, 2005). Field-scale studies at SAC compared certified seed with untreated home-saved contaminated with *R. secalis* (Oxley *et al.*, 2008). This work demonstrated that seed-borne infection can lead to widespread development of rhynchosporium symptoms. Where the weather was ideal for disease development following this initial outbreak (cool and wet), the difference in disease severity

between dirty and clean seed continued over the following months (Figure 1). Researchers are looking for the presence of airborne spores associated with a potential ascospore stage of *R. secalis*, but these field studies showed that the uniform presence of rhynchosporium symptoms in a field crop can be associated solely with contaminated seed.

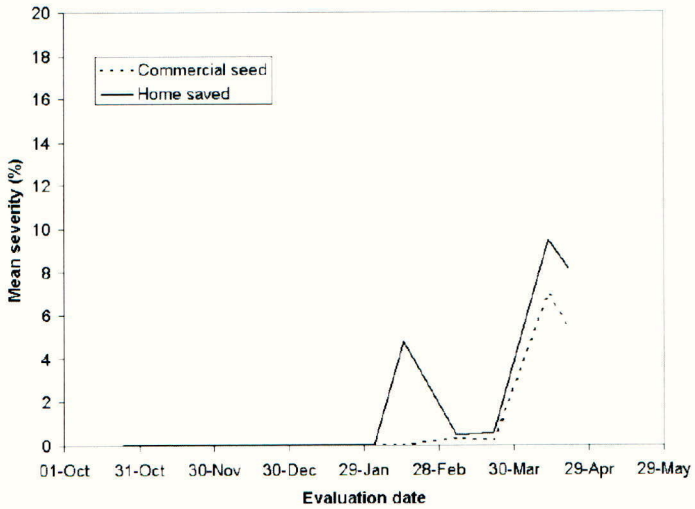

Figure 1 Rhynchosporium symptom development in 2005 for clean commercial and contaminated home-saved seed.

Seed movement

The presence on seed of fungal pathogens that can cause foliar diseases raises issues regarding the risk of spreading new races of *R. secalis* and *R. collo-cygni* to different regions or countries. Movement of foliar pathogens on barley seed may be a more immediate threat to spreading new pathogen races than the risk of changes in disease patterns occurring as a consequence of climate change.

Spring barley plant breeders have nurseries in the UK, mainland Europe and also New Zealand. This provides major advantages to breeders by providing them with two seasons each year to study breeding material, but there are risks of importing pathogens adapted to mainland Europe or New Zealand to the UK through seed movement. This could lead to an increased risk of importing pathogen races adapted to different environmental conditions.

Movement of commercial seed stocks from one region to another is likely to be a greater risk of spreading unwanted foliar pathogens. *Rhynchosporium secalis* resistant to quinone outside inhibitor fungicides (QoIs) was found in France in 2008 and reported by the Fungicide Resistance Action Committee.

Although QoI resistance may occur in other regions independently, the added risk of importing fungicide resistance from one region to another is not fully understood, but seed produced in France from regions affected by this resistance and subsequently exported could potentially spread fungicide resistance at a faster rate than would occur otherwise.

Seed treatments and legislation

The European Parliament voted in January 2009 to accept revisions to EU directive 91/414 EEC which will lead to the exclusion of substances with a very hazardous profile over a 10–15-year period. Risk assessments carried out by the Pesticide Safety Directorate and the Swedish Chemicals Agency indicate that the industry may lose many important cereal seed treatments and foliar fungicides. Should this happen, seed health will become a more important factor in the management of some important foliar pathogens.

Conclusions

Molecular diagnostics is an effective tool to increase our understanding of the epidemiology of barley pathogens. Since seed contamination plays a major role in early disease epidemics of rhynchosporium, and seed transmission is one of the main methods to spread ramularia leaf spot, should more importance be placed on seed health associated with these diseases? Changes in the availability of some of the main fungicides available to manage diseases, applied either as seed treatments or foliar fungicides, will place greater importance on the health of barley seed, and will also require more monitoring of seed movement to prevent the spread of new populations of rhynchosporium resistant to QoI fungicides or spreading ramularia to regions where it has yet to become an established disease of major economic importance.

Acknowledgements

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Spring cereal seed infected with *Microdochium nivale*: cause for concern?

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Summary

Exceptionally high levels of *Microdochium nivale* were recorded on spring cereals seed from the 2007 harvest. Small-scale experiments consistently suggested that infection levels up to 50% would have limited effect on spring barley sown into spring seed beds. Laboratory experiments in 2008 showed potential risks for untreated spring wheat when sown into cold seed beds, but suggested oats were less of a risk. A field experiment sown early and late April 2008 confirmed there was a high risk for spring wheat with *M. nivale* seed infections of 30%. Spring oats were also at risk but the results were more variable in the second sowing. A seed lot of spring barley infected with 58% *M. nivale* showed a 24% seedling loss in the early sowing. A second sowing of the same seed lot 2 weeks later showed no seedling losses. High levels of *M. nivale* have the potential to affect all spring cereals. Data suggest that untreated spring barley is only at risk where infection levels are very high.

Introduction

In the UK, seedling blight, caused by *Microdochium nivale* (Fr.) Samuels & I.C. Hallett, is known to reduce emergence in winter wheat and winter oats when untreated seed is sown. However, the severity of seedling blight is dependent on the level of seed-borne infection and on a number of factors including soil temperature and soil moisture which influence the rate of seedling emergence. The 'worst case' relationship between *M. nivale* seed infection and emergence in winter wheat has been quantified by Cockerell *et al.* (2004) from a series of late-sown field experiments. A 10% threshold was calculated, above which the benefits of seed treatment would be cost-effective where late sowing or seedbed conditions delay emergence. No threshold has been calculated for winter oats. Winter barley and spring cereals are considered to be at less of a risk from *M. nivale* seedling blight as seed bed conditions are less conducive for transmission of infection. Barley is also thought to be less susceptible to *M. nivale* infection than wheat or oats as losses due to seedling blight are rare. Spring cereal seed harvested in 2007 and tested at the Official Seed Testing Station for Scotland (OSTS) showed a very high incidence of *M. nivale* seed infection. Average seed infection for spring barley, spring wheat and spring oats was 45, 30 and 24%, respectively. Sixty-six per cent of spring barley samples had seed infection levels greater than 50%, and 26% of spring barley samples had greater than 70% seed infection. More than a quarter of the spring wheat and oats tested had greater than 50% seed infection. For all spring cereals these levels were higher than the previous 4 years, when average levels were below 10% seed infection with only an occasional sample above 50%. Lack of information available on the effect of *M. nivale* on spring cereal emergence made it difficult for seed growers to interpret these very high infection levels. Although seed treatment provided an option for conventional growers, for organic growers

such high results only provided uncertainty. This paper reviews the results from laboratory and field experiments conducted at the OSTS in seasons 1981/82, 1992/93, 1994/95 and 2007/08 to determine the potential effect of seed-borne *M. nivale* on spring wheat, oats and barley.

Method

Seed lots

Untreated spring cereal seed lots with a range of *M. nivale* infection levels were chosen from samples submitted to the OSTS for testing. Experiments were conducted in 1994/95 or 2007/08. Details of lots and experimental year are given in Table 1.

Table 1 Seed lot, harvest year, variety and percentage *Microdochium nivale*

Seed lot	Season	Type	Variety	% *M. nivale*	Pot experiment	Field experiment
1	1980/81	Spring barley	Golden promise	6		✓ (single rows)
2	1980/81	S. barley	Triumph	17		✓ (single rows)
3	1980/81	S. barley	G. promise	44		✓ (single rows)
4	1980/81	S. barley	G. promise	51		✓ (single rows)
5	1992/93	S. barley	Derkado	3		✓
6	1992/93	S. barley	Derkado	50		✓
7	1992/93	S. oat	Unknown	54		✓
8	1994/95	S. barley	Derkado	53	✓	
9	1994/95	S. barley	Derkado	2	✓	
10	2007/08	S. wheat	Chablis	69	✓	
11	2007/08	S. wheat	Chablis	32	✓	✓
12	2007/08	S. wheat	Paragon	47	✓	
13	2007/08	S. wheat	Paragon	8	✓	
14	2007/08	S. oat	Firth	28	✓	
15	2007/08	S. oat	Firth	18	✓	
16	2007/08	S. oat	Firth	5	✓	
17	2007/08	S. wheat	Paragon	0		✓
18	2007/08	S. wheat	Tybalt	31		✓
19	2007/08	S. oat	Atego	11		✓
20	2007/08	S. oat	Firth	36		✓
21	2007/08	S. oat	Firth	31		✓
22	2007/08	S. barley	Cocktail	40		✓
23	2007/08	S. barley	Waggon	41		✓
24	2007/08	S. barley	Optic	58		✓

Germination tests

Germination tests were conducted in accordance with the International Seed Testing Association Rules (ISTA rules) rolled-paper towel method. To break dormancy, a cold pre-treatment at 7 ± 2°C was used prior to growth at 20 ± 0.5°C.

Pot experiment

Four replicates of 100 seeds (barley lots 8 and 9) or 50 seeds (wheat, lots 10–13 and oats, lots 14–16) from each untreated sample were sown into a 200 mm pot containing J Arthur Bowers (1994) or John Innes No. 2 (2007) compost. Sub-samples of the two barley lots were also sown treated with Beret gold. Seeds were sown at a depth of 50 mm and the soil was adjusted to a field capacity of 75%. The pots were placed at 7 ± 2°C for 19 days (barley) and 23 days (wheat and oats). Seedling emergence was counted daily until maximum emergence was observed. The field capacity was maintained throughout the 19–23-day period by adding water as required. After this period, pots were transferred to a controlled temperature room at 15–20 ± 1°C for 9–10 days and further emergence noted. At the end of the trial, seedlings in the pots were assessed in accordance with ISTA rules. In addition to normal and abnormal categories, the seedlings were further assessed for *M. nivale* symptoms according to Table 2.

Field experiments

Small-scale field experiments were conducted in each of years 1981, 1993 and 2008. In 1981, four seed lots (1–4) were sown out in single rows both untreated and treated with organomercury (Panogen) on 25 March 1981 at East Craigs, Edinburgh. Eight replicates of 50 seeds were sown per treatment. In 1993, two lots of barley (5 and 6) and one oat (7) were sown out untreated and treated with guazatine + imazalil (Rappor plus). Plots 4 m^2 were sown at a target rate of 350 seeds per m^2 in a randomised complete block design. Similarly, in 2008 three lots of wheat (11, 17 and18), three lots of oats (19, 20 and 21) and three lots of barley (22, 23 and 24) were sown out untreated and treated with fludioxinil (Beret gold). Plots 8 m^2 were sown at a target rate of 400 seeds/m^2 in a randomised complete block design. All treatments were used at the manufacturer's recommended rate. All seedling counts were made at growth stage 11–12. Percentage seedling loss due to sowing untreated seed with *M. nivale* infection was calculated as a percentage of treated seed plant populations.

Table 2 Seedling disease assessments

Category	Seedling description	Score
Healthy	No disease symptoms	1
Low infection	Slight browning on coleoptile	2
Medium infection	Whole coleoptile browned or root browning	3
High infection	Damage not just superficial on coleoptile but through to stem tissues	4

Results

Pot experiments

There was no significant difference in emergence of barley or oat lots tested due to *M. nivale* infection (Table 3, Figure 1). Low emergence for the Firth oat lot (16) with a low level of *M. nivale* was due to factors other than *M. nivale*. The standard germination test for this sample was 77% compared with 92 and 89% for oat lots 15 and 14, respectively. The emergence for spring wheat decreased as the *M. nivale* level increased (Figure 1). Mean emergence time for barley and wheat increased as levels of *M. nivale* increased. This was not the case for oats, where lot 16 Firth with lowest *M. nivale* had the highest mean emergence time.

The average disease levels found on seedlings were higher as *M. nivale* levels increased for wheat and barley (Figure 2). However, most seedlings in category 4 (Table 2) for barley samples were normal (in terms of germination assessment) whereas for wheat most seedlings in category 4 were abnormal and would not produce a seedling in the field. Seedling disease levels for oats did not increase with *M. nivale*. Sample 16 Firth with the lowest infection level had the highest disease score for oats. This was not a result of *M. nivale* infection but appeared to be related to damping off.

Field experiments

Four spring barley lots sown out in a single row experiment in March 1981 showed no decrease in emergence with increasing levels of *M. nivale* infection (Table 4). In 1993, untreated spring barley and spring oat seed lots sown in experimental plots with 50 and 54% *M. nivale*, respectively, showed a small decrease in emergence compared with the treated plots (Table 4). However, the untreated emergences when compared with the original laboratory germinations for both lots were not significantly different. High seedling losses (>30%) were seen in plots sown on 4 April 2008 when *M. nivale* levels were above 30% in both spring wheat and spring oats (Figure 3). A high seedling loss (24%) was recorded only when *M. nivale* seed infection was 58% (spring barley lot 7). Significant seedling losses for spring wheat above 30% were also seen in the second sowing (22/4/08) (Figure 3). Losses for spring oats were more variable with an 18% loss for lot 11 (31% *M. nivale* seed infection) compared with no seedling loss in lot 10 (36% seed infection). No significant losses were recorded for spring barley in any seed lot.

Table 3 Maximum emergence, seedling disease score and mean emergence time for two barley seed lots infected with *M. nivale* sown in pots with compost held at 75% field capacity

Seed lot/ treatment	Germination test result	Percentage *M. nivale*	Maximum emergence	Mean emergence time (days)	Seedling disease score
8. Untreated	96	53	91.25	22.7	2.90
8. Treated	94	53	93.00	24.8	1.02
9. Untreated	98	1.5	96.00	21.6	1.10
9. Treated	97	1.5	98.00	22.5	1.01

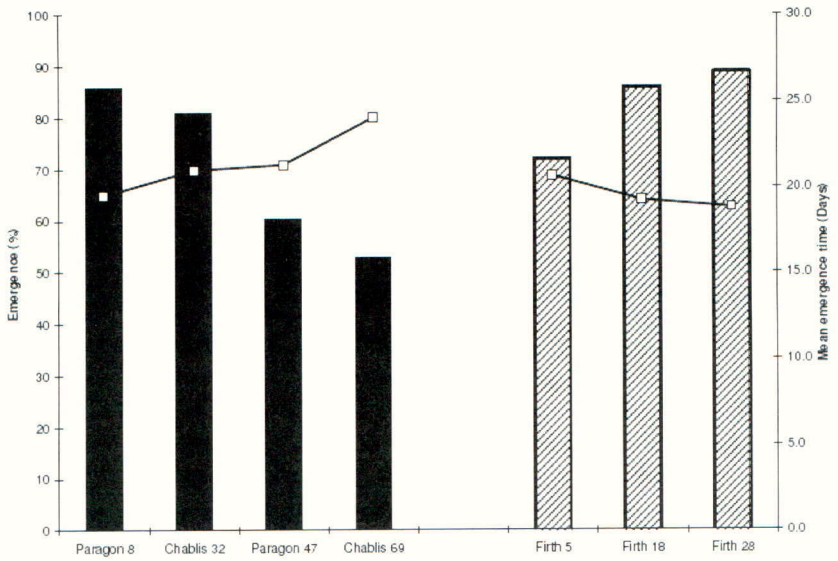

Figure 1 Maximum emergence (columns) and mean emergence time (line) for wheat and oat seed lots infected with *Microdochium nivale* sown in pots with compost held at 75% field capacity

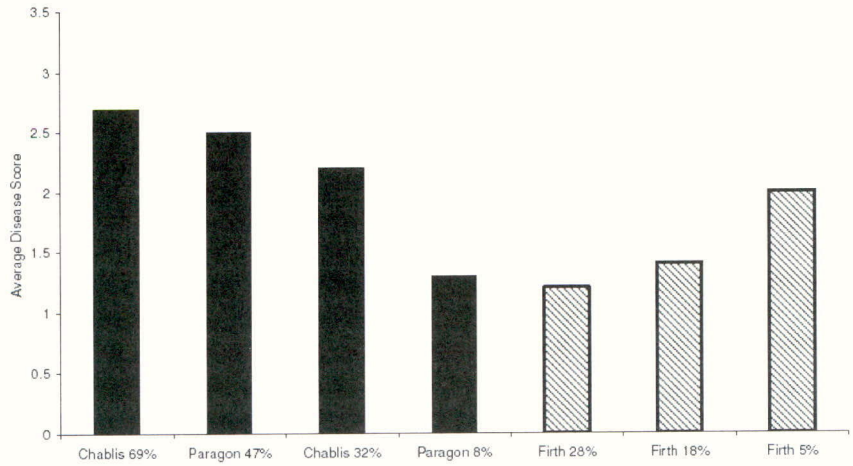

Figure 2 Average disease scores for wheat and oat seed lots infected with *Microdochium nivale* sown in pots with compost held at 75% field capacity

Table 4 Mean field emergence results for untreated and treated barley and oat, 1981 and 1993

	1981 single row sowings				1993 field sowings		
Seed lot	**1 B**	**2 B**	**3 B**	**4 B**	**5 B**	**6 B**	**7 O**
% *M. nivale*	6	17	44	51	3	50	54
Emergence (Un)	98	93	93	91	92	88	92
Emergence (Tr)	98	94	95	93	81*	100	100
Germination Test	99	97	94	96	94	94	98

B = barley; O = oat.

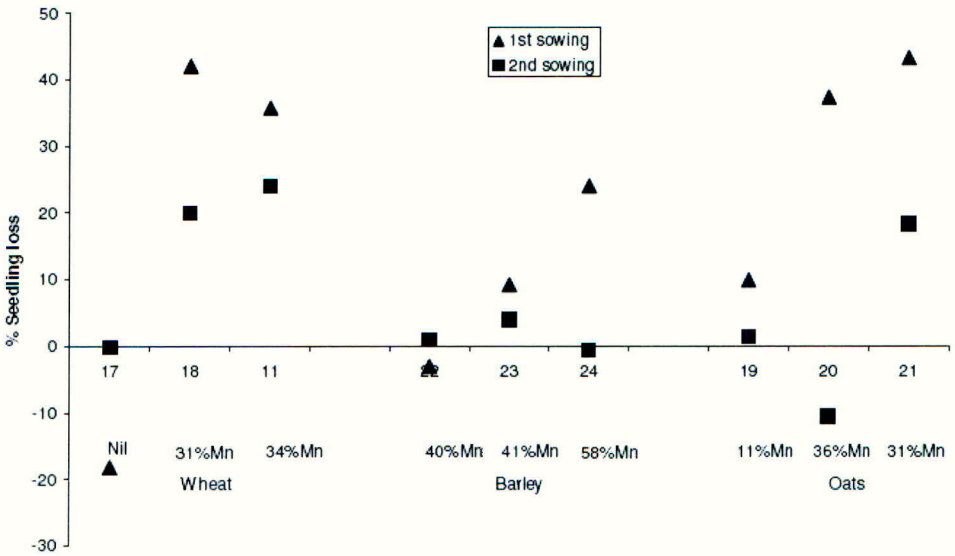

Figure 3 Mean seedling loss for spring wheat, spring barley and spring oats sown 4 April 2008 and 22 April 2008 at Gogarbank Farm, Edinburgh

Discussion

Microdochium nivale has been reported to cause pre-emergence and post-emergence death of seedlings, and ear infection in winter sown barley, wheat and oats. Richardson *et al.* (1976) found that spring barley infected with high levels of seed infection did not show a reduction in germination, but infection was associated with higher levels of seedling disease if seed was sown untreated. Although high levels of seed infection are recorded on some spring barley samples, infection was not associated with reduced emergence until spring 2008. Data from sowings in 1981, 1993 and pot experiments in 1994/95 supported the view that *M. nivale* was not of concern for spring sowing. However, field sowings in 2008 showed that a high infection level of 58% *M. nivale* seed infection caused significant seedling losses where untreated seed

was sown in early April. The same seed lot sown 2 weeks later caused no seedling losses. It is possible that the unusually cold spell in the week after sowing encouraged the expression of seedling blight in this case. Early data presented for spring oats suggest that, like barley, they appeared to be less susceptible to *M. nivale* infection. Pot experiments showed no evidence of high seedling losses due to *M. nivale* up to levels of 28% infection. Spring wheat, on the other hand, showed increased seedling loss with increased *M. nivale* seed-infection levels in both pot experiments and field experiments in 2008. Unlike barley, both the wheat and oat samples showed reduced emergence in both sowings, although oats were more variable in their response. Based on these limited data sets, it can be concluded that spring wheat and oats are at risk from high levels of *M. nivale* infection and spring barley is at risk, but at levels exceeding 30% seed infection.

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Relationship between seedling emergence in winter wheat and levels of *Microdochium nivale* DNA determined by real-time PCR

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Summary

Seedling emergence counts were carried out in field experiments using seed lots with a range of *Microdochium nivale* infection levels, over three seasons. Comparison of seedling loss and ng DNA results from a real-time PCR method showed a significant relationship in 2 out of 3 years ($P = 0.001$ and $P = 0.005$). However, seedling loss in some samples with high DNA levels were lower than expected. Single seed extractions confirmed the heterogeneity of *M. nivale* distribution in the seed lot resulting in the potential of one seed contributing a high proportion of the DNA measured in the PCR test but only contributing to seedling loss for one seedling. At present using DNA levels to determine a seed treatment threshold would not improve the interpretation of results compared with current procedures based on a threshold set using the agar plate test.

Introduction

In the UK, *Microdochium nivale* (Fr.) Samuels & I.C. Hallett is the most common pathogen of winter wheat involved in seedling blight. An HGCA study on cereal seed health and seed treatment strategies advised a treatment threshold of 10% infection, above which the benefits of seed treatment would be cost-effective (Cockerell *et al.*, 2004). The threshold was based on a comparison of *M. nivale* seed lot infection, determined using an agar plate test (Cockerell, 2009), against seedling loss in a series of field experiments. Field experiments were sown late to achieve, as far as possible, maximum disease expression. A real-time PCR test for *M. nivale* seed infection has been offered in the UK since 2004. Interpretation of the test results is based on the relationship between the level of *M. nivale* DNA, measured by PCR, and percentage seed infection, measured in the agar plate test over a number of calibration data sets. Adjustments have been made over seasons using a Bayesian statistical approach. There is little known about the relationship between DNA loading (ng DNA), obtained from the real-time PCR assay, and seedling emergence. Understanding this relationship in infected seed lots could allow for interpretation of results to be based solely on the PCR test rather than the complicated statistical approach currently used. This paper describes the field experiments set up to establish this relationship.

Method

Seed lots

In each of the 3 years a selection of samples from naturally infected seed lots with a range of *M. nivale* infection levels were obtained from Scottish wheat growers (Table 1).

Table 1 Seed lots, variety, agar plate test results and real-time PCR results

	2004/05			2005/06			2006/07		
Lot	**Variety**	**% Mn**	**ng Mn DNA**	**Variety**	**% Mn**	**ng Mn DNA**	**Variety**	**% Mn**	**ng Mn DNA**
1	Savannah	0	29.5	Einstein	0	50.1	Robigus	0	13.5
2	Savannah	0.5	31.6	Consort	0.5	3.1	Alchemy	0	0.14
3	Consort	3	67.6	Robigus	1	33.1	Alchemy	0.5	27.5
4	Riband	6.5	199.5	Malacca	1	104.7	Consort	0.5	33.1
5	Pegassus	7.5	67.6	Robigus	2	47.9	Robigus	7	63.1
6	Malacca	18	234.4	Riband	4.5	49.0	Alchemy	13	275.4
7	Consort	19	501.2	Robigus	4.5	131.8	Claire	15.5	346.7
8	Consort	20	691.8	Malacca	5.5	281.8	Alchemy	18.5	245.5
9	Robigus	22.5	489.8	Nijinsky	10	389.1	Alchemy	27	245.5
10	Robigus	25	467.7	Robigus	17	467.7	Robigus	28	182.0
11	Robigus	27	575.4	Consort	18	794.3	Robigus	35.5	309.0
12	Robigus	32	645.7	Robigus	19	776.2			
13	Robigus	33.5	812.8	Predator	20.5	1000.0			
14	Robigus	66.5	1445.4	Robigus	26	758.6			
15				Robigus	33	933.3			

Seed testing and seed treatment

Each seed lot was thoroughly mixed and then divided into two sub-samples. One sub-sample was left untreated and the other was treated with Sibutol® (bitertanol & fuberidazole) at the recommended rate, using a Rotostat seed treatment machine. Each treated sub-sample was tested for germination and the untreated portions were tested for: *M. nivale* infection; tetrazolium; moisture; thousand seed weight (Anon., 2009) and ng *M. nivale* DNA (Cockerell *et al.*, 2004).

Field experiment

For each treatment plot samples were prepared using the thousand seed weight, to calculate the quantity of seed required, providing a target seed rate of 450 seeds/m^2. Seed was drilled into 10 × 1 m plots. The plots were sown in a randomised block design with four replicates. Plots were sown late (11/11/2004, 6/11/2005 and 6/11/2006) to ensure symptom expression in each year, and emergence counts were made the following January at the first leaf stage on 5 × 1 m rows in each plot.

Single seed analysis

DNA extractions from 50 single seeds were prepared using an extraction method that incorporates a CTAB extraction described by Edwards *et al.* (2001), from a sample with an infection level of 9% *M. nivale* ascertained by agar plate test. These extractions were tested using the real-time PCR method to obtain the level of *M. nivale* inoculum present on each individual seed in ng DNA.

Results

Seed testing

Tetrazolium (viability), germination and moisture results confirmed the suitability of samples for use in the field experiments. Agar plate test and real-time PCR results are given in Table 1. In each of the 3 years there was a good relationship between percentage *M. nivale* seed infection and ng *M. nivale* DNA, R^2 = 0.9364, 0.8807 and 0.6413 for each of the three years, respectively.

Field experiment

The mean emergence counts (plants/m^2) for untreated and treated plots are presented in Figure 1. Differences were seen between untreated and treated plots in years 2004/05 and 2005/06, with lower emergence in untreated plots at high levels of *M. nivale* seed infection. Data for 2006/07 show poor emergence in both untreated and treated plots. High rainfall leading to flooding of the plots and higher than expected temperatures resulted in very poor emergence. No differences as a result of *M. nivale* seed infection were found in this year.

The percentage seedling loss due to *M. nivale* was calculated using the difference between the untreated and treated populations as a percentage of the treated population. Seedling loss plotted against ng DNA for years 2004/05 and 2005/06 is shown in Figure 2. Although the relationship was significant in years 2004/05 (P = 0.001) and 2005/06 (P = 0.005), the

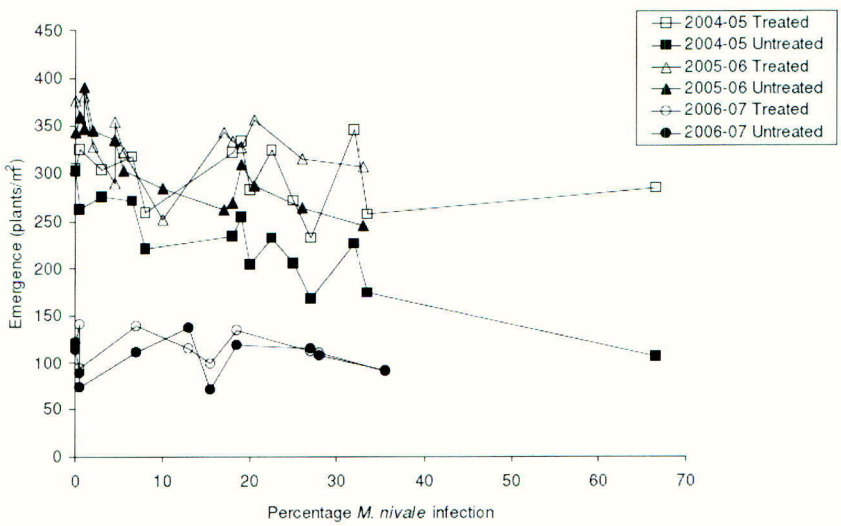

Figure 1 Mean untreated and treated emergence counts (plants/m^2)

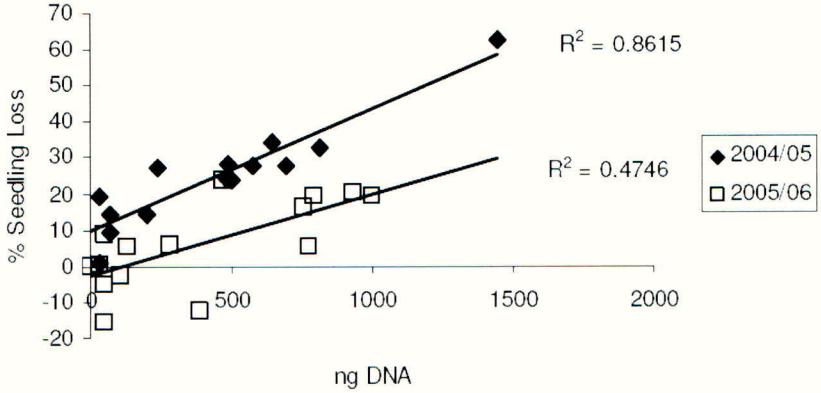

Figure 2 Percentage plant loss 2004/05 and 2005/06 compared with ng DNA *Microdochium nivale*

results were more variable in the second year where there was a higher number of lots with low *M. nivale* infection levels.

Single seed analysis

Microdochium nivale DNA was detected in all extracts prepared from the individual seeds. The concentration of DNA varied greatly from one seed to another (Figure 3). In some seed extracts it was present as a trace, in others over 100 ng were detected, with a maximum of 2531 ng *M. nivale* DNA.

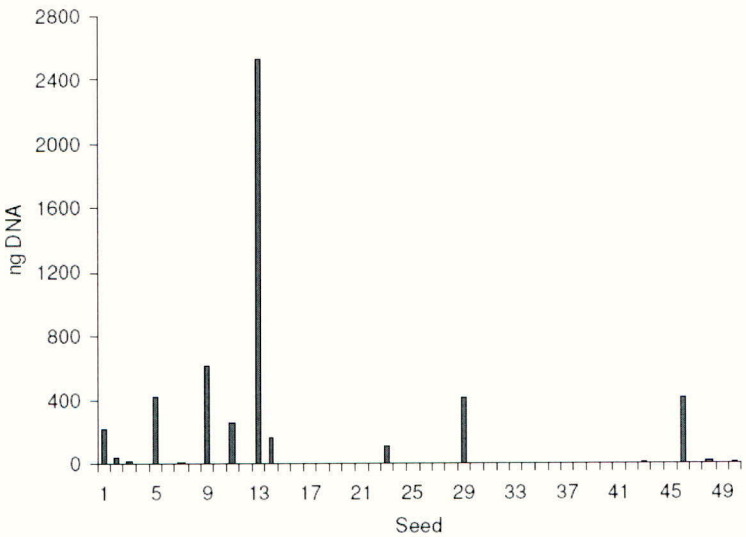

Figure 3 Single seed extracts showing the different magnitudes of inoculum loading on individual seeds

Discussion

The severity of seedling blight is dependent on the level of seed-borne infection and on a number of factors, including seed bed condition, soil temperature and soil moisture, therefore the relationship between levels of *M. nivale* and seedling establishment over the 3 years has been mixed. That said, field experiments in 2004/05 and 2005/06 have shown that there is a good relationship between quantitative real-time PCR results and seedling establishment.

However, the variability of *M. nivale* DNA levels at low *M. nivale* seed infection as determined by the agar plate test, particularly in year 2005/06, meant it was difficult to determine the level of DNA at which seed treatment would be advisable (based on the current threshold of 10% seedling loss).

Analysis of single seed extractions showed great variation between *M. nivale* loading of individual seeds. Whilst very low levels were detected on the majority of seeds, occasional seed extractions produced spikes of inoculum. It is likely that much of the variability seen in the field experiment could be due to the heterogeneous nature of *M. nivale* inoculum on individual seeds within the lot. This highlights a major difference between the two test methods for detecting *M. nivale*. The agar plate test measures percentage of infection by examining 200 individual seeds – either the seed is infected, or it is not. The real-time PCR test determines the amount of DNA in a group of 200 seeds and, provided the extract is homogeneous, an average inoculum level is reported. In the agar plate test, one infected seed would give rise to

Table 2 Ranking of results from agar plate and real-time PCR tests

Rank	2004/05 % infection	2004/05 ng DNA	2005/06 % infection	2005/06 ng DNA
1	0	29.7	0	50.1
2	0.5	31.3	0.5	3.1
3	3	67.7	1	33.1
4	6.5	198.7	1	104.7
5	8	67.4	2	47.9
6	18	236.3	4.5	49.0
7	19	496.6	4.5	131.8
8	20	695.3	5.5	281.8
9	22.5	488.2	10	389.1
10	25	473.2	17	467.7
11	27	575.7	18	794.3
12	32	648.1	19	776.3
13	33.5	820.8	20.5	1000.0
14	66.5	1435.3	26	758.6
15			33	933.3

one infected plant in the field. In the PCR assay, one heavily loaded seed could contribute the majority of the DNA detected in the seed sample, and yet still account for only one infected seedling in the field. This means that using this test method, there is a risk that infection levels may be predicted at a higher level than in the agar plate test.

To investigate further differences in the relationship between seedling loss and ng *M. nivale* DNA, the relationship between agar plate test (percentage infection) and real-time PCR (ng DNA) results was examined in more detail. When seed lot results were ranked according to percentage *M. nivale* infection, it was apparent that for ng DNA ranking of some samples would be different (Table 2). For example, in year 2004/05, a 20% infection sample ranked 8th for *M. nivale* by agar plate test, would be ranked 12th for ng DNA by real-time PCR.

Conclusions

Results from quantitative real-time PCR assay do correlate significantly with seedling emergence in the field, making the real-time PCR assay a useful tool for estimating seedling loss. However, the heterogeneous loading of DNA on individual seeds means that definition of a threshold based on DNA levels would also require the development of statistical models to interpret the results, and the risks that already exist with regard to false positives in relation to the threshold would remain.

References

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