$$P_{+} = P_{cont} \times P_{d}$$

Arbitrarily, an unacceptable seed lot was defined as one in which the expected average contamination of transplants was greater than 10% at the time of planting (6 weeks after sowing), and an unacceptable test was indicated when the probability of detection was less than the probability of transmission for an unacceptable lot.

Results and conclusions

Some example scenarios are shown in Table 1, starting with seed infestation levels ranging from 1 in 5,000 to 1 in 50,000 seeds and mean numbers of *Xcc* per infested seed from 10 to 1000. The remaining columns show the results of running the transmission and spread models, together with the probabilities of obtaining a positive seed test result with and without a centrifugation step.

The transmission and spread models suggested that the high levels of disease incidence often seen in the field can be explained by rapid rates of pathogen spread during plant-raising, and

Table 1 Example scenarios for different proportions of infested seed and numbers of *Xanthomonas campestris* pv *campestris* per infested seed, together with the probability of a positive test result with (Cent) and without (No cent) centrifugation to improve analytical test sensitivity

One infested seed in:	% infested	CFU per infested seed	Probability of transmission	Average % contamination of transplants	Probability of positive seed test	
					Cent.	No cent.
50,000	0.002	10	0.06	0–5	0.08	0.01
		100	0.12	1–11	0.39	0.08
		1000	0.23	1–21	0.45	0.39
25,000	0.004	10	0.14	1–13	0.13	0.01
		100	0.26	3–26	0.60	0.13[1]
		1000	0.47	5–46	0.70	0.60
10,000	0.01	10	0.25	7–25	0.17[1]	0.02[1]
		100	0.46	12–45	0.82	0.17[1]
		1000	0.72	19–71	0.95	0.82
5,000	0.02	10	0.44	20–44	0.33[1]	0.04[1]
		100	0.71	32–70	0.98	0.33[1]
		1000	0.92	42–91	0.99	0.98

[1]Unacceptable tests.

that the widely used tolerance standard for seed health testing (0.01%) is inadequate and should be revised to 0.004%. Given the potential difficulty of achieving this standard (it requires 75,000 seeds to be tested), in addition to seed health testing, control should focus on raising transplants under conditions that minimise the rate of disease/pathogen spread.

The results also indicated that omitting the centrifugation step (as in the current ISTA method) gives a greater risk of unacceptable tests. The greatest danger of detection failures occurs with seed lots with a relatively high percentage infestation but low numbers of bacteria per seed, and highlights the importance of both the detection limits and analytical sensitivity when designing effective seed health assays.

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Occurrence and importance of seed-borne *Bipolaris sorokinana* in Norwegian barley

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Summary

During recent years *Bipolaris sorokiniana* has occurred at high frequencies in seed lots of the barley cvs Annabell and Edel in Norway. In seed treatment experiments with two heavily infected barley lots the infection level was reduced by chemical treatment and field emergence and yields were significantly increased. The best effect was seen with an imazalil + flutriafol compound which increased the yield by approximately 35% compared with untreated. A healthy untreated seed lot of the same cultivar showed approximately the same level of emergence and yield as the best fungicide treatment of a heavily infected seed lot.

Introduction

Bipolaris sorokinana (teleomorph *Cochliobolus sativus*) is a widespread pathogen of cereals and many grasses. It can infect seeds, roots and leaves, causing seedling blight, common root rot, foot rot and spot blotch. Inoculum of *B. sorokiniana* may be seed-borne or arise from infected plant debris in the field and from conidia in the soil. Under favourable conditions infections may result in severe yield losses, due to reduced stand establishment, reduced tillering and shrivelled kernels with reduced size and weight. The pathogen has been considered to be most important in barley and wheat in warm temperate areas. However, it has also been reported to be important in cool climates of Northern Europe (Olofsson, 1976; Kurppa, 1984; Jørgensen, 1986).

In Norway, all cereal seed lots are tested for seed-borne pathogens (Brodal, 1993) and for many years *B. sorokinana* has been observed only sporadically in barley, oats and wheat seeds. However, during recent years the pathogen has been recorded at rather high frequencies in seed of barley, especially the cultivars Edel and Annabell. It was decided to include routine testing for *B. sorokinana* in all seed lots of these two cultivars from 2004. Cultivars Frisco and Helium were also included from 2006. The incidence of *B. sorokinana* recorded in these barley cultivars is presented below. In order to evaluate the importance of the seed-borne inoculum in barley and to test the effect of seed treatment fungicides against the pathogen, field and laboratory experiments have been carried out. Results from experiments in 2007 are presented.

Materials and methods

Occurrence of B. sorokinana in seed

The number of seed lots tested for *B. sorokinana* from 2004 to 2008 is shown in Table 1, and included both seed intended for certification and farm-saved seed. The presence of the fungus was determined after 4 days incubation at 10°C followed by 4 days at 20°C on moist filter paper, according to a modified version of the Doyer method (Jørgensen, 1971). After incubation, the seeds were examined individually under a stereomicroscope (6–25×) and the number of seeds showing sporulation of *B. sorokinana* recorded as infected. Of each sample, 100 seeds were tested.

Seed treatment experiments

Two field experiments were established in 2007. Seed from two naturally infected seed lots (Annabell, 90% infection; Edel, 40% infection) were treated with fludioxinil (Celest 025 FS), guazatine + imazalil (Panoctine Plus), imazalil + flutriafol (Fungazil Gold) and *Pseudomonas chlororaphis* (Cedomon) at recommended doses. The four treatments and an untreated control were sown in field plots of 1.5 × 8 m in three replicates (randomised block design). In addition, healthy seed (as healthy as possible) of the same cultivar was included in each experiment as a healthy control. Samples from all treatments and controls were tested in the laboratory for germination and presence of *B. sorokiniana*. In the field, emergence was recorded at growth stage BBCH 12–13 by counting number of seedlings in 4 × 1 m row in the plot. Plots were harvested and the yield measured. Seed samples from the harvested yield of each plot were tested in the laboratory for the presence of *B. sorokiniana*.

Results

Occurrence of B. sorokinana in seed

A large proportion of the seed lots tested for *B. sorokiniana* were infected, and the average infection frequencies were rather high (Table 1). Despite this, the germination capacity (results not shown) were in general not severely affected. Most of the seed lots showed a germination percentage above the minimum requirements of 85% for certification.

Seed treatment experiments

In both experiments, chemical seed treatment reduced the infection level in the seed and increased emergence in the field (Tables 2 and 3). The best effect was found with compounds containing imazalil. Imazalil + flutriafol treatment showed better emergence than guazatine + imazalil with the most infected seed lot (Annabell, Table 2). Both imazalil + flutriafol and guazatine + imazalil significantly increased the yield compared with untreated seed in the most infected seed lot, Annabell. Only imazalil + flutriafol treatment showed significant yield effect in the seed lot of Edel (Table 3). No increased emergence or significant yield increase was found after treatment with *Pseudomonas chlororaphis*.

Emergence and yield in the healthy untreated seed was approximately the same as the most effective seed treatments.

Laboratory tests of seed harvested from all treatments showed a high and consistent level of between 92 and 97% seeds infected with *B. sorokiniana* (data not shown). This indicates

Table 1 Incidence of *B. sorokiniana* in seed lots of the barley cultivars Annabell, Edel, Frisco and Helium grown in Norway during 2004–08

Cultivar	Year of harvest	Number of samples tested	Percentage of samples infected	Average infection frequency
Annabell	2004	88	26	3.4
	2005	148	45	7.8
	2006	123	96	30.7
	2007	94	90	62.5
	2008	41	100	56.0
Edel	2004	382	97	13.6
	2005	527	94	17.0
	2006	444	98	27.9
	2007	362	99	52.3
	2008	243	99	20.2
Frisco	2006	13	84	38.9
	2007	9	100	55.9
	2008	6	83	8.1
Helium	2006	43	77	4.8
	2007	69	75	4.7
	2008	108	69	2.1

that the inoculum of this pathogen can easily spread from infected to healthy plots during the growing season.

Discussion

The high incidences of *B. sorokiniana* in certain cultivars indicate that there are rather clear differences in susceptibility among barley cultivars grown in Norway. The importance of resistant cultivars has been discussed by Piening (1997) and Steffenson (1997).

The investigations indicate that the use of healthy seed, or seed treated with an effective fungicide, is important to reduce the damage from *B. sorokiniana*.

Table 2 Germination (%), incidence of *B. sorokiniana* (%), emergence (number of seedlings/m row) and yield (kg/ha) in a seed treatment experiment in Norway 2007 using naturally infected seeds of barley cv. Annabell

Seed lot	Treatment	g a.i./kg seed	Dose (ml/kg)	Laboratory		Field		
				% germination	% infection	No. of seedlings	Yield (kg/ha)	Relative yield
1 (healthy)	Untreated			85	17	62	3480	100
2 (infected)	Untreated			95	89	44	2620	75
	Fludioxinil	0.05	2	95	33	54	2920	84
	Pseudomonas chlororaphis		7.5	94	86	44	2910	84
	Imazalil + guazatine	0.04 + 0.6	2	93	15	58	3180	91
	Imazalil + flutriafol	0.05 + 0.04	2	96	5	71	3630	104
Lsd 5%					5.4	11.4	450	

Table 3 Germination (%), incidence of *B. sorokiniana* (%), emergence (number of seedlings/m row) and yield (kg/ha) in a seed treatment experiment in Norway 2007 using naturally infected seeds of barley cv. Edel

Seed lot	Treatment	g a.i./kg seed	Dose (ml/kg)	Laboratory		Field		
				% germination	% infection	No. of seedlings	Yield (kg/ha)	Relative yield
1 (healthy)	Untreated			97	1	75	3330	100
2 (infected)	Untreated			89	40	53	2390	72
	Fludioxinil	0.05	2	97	15	60	2880	87
	Pseudomonas chlororaphis		7.5	92	37	55	2260	68
	Imazalil + guazatine	0.04 + 0.6	2	93	6	66	2490	75
	Imazalil + flutriafol	0.05 + 0.04	2	95	1	67	3110	93
Lsd 5%					3.2	7.3	490	

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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.

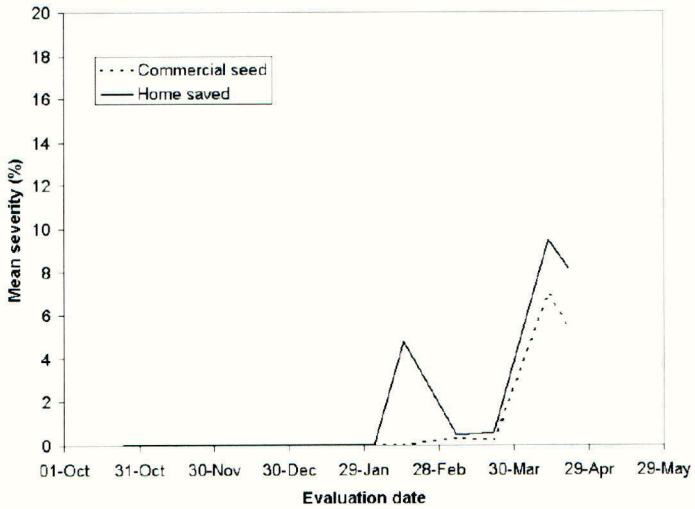

Figure 1 Rhynchosporium symptom development in 2005 for clean commercial and contaminated home-saved seed.

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