Cereal Seed Health and Seed Treatment Strategies: Exploiting new seed testing technology to optimise seed health decisions for wheat.

Technical Paper No. 4

The effect of *Microdochium nivale,* on the quality of winter wheat seed in the UK.

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INTRODUCTION

Microdochium nivale (*Fusarium nivale*) causes pre-emergence blight of seedlings of winter wheat. *M. nivale* is both seed- and soil-borne. Seed-borne inoculum is the most important source of infection. Although it is present in the soil and can survive on straw and plant debris, Paveley and Davies, 1994 have shown that the risk from soil-borne inoculum in UK field soils is low. Both Cockerell (1995) and Humphreys *et al.* (1995) have shown that the emergence of untreated winter wheat seed is related to levels of *M. nivale* on the seed. As seed infection increases emergence decreases. However the relationship between *M.nivale* infection and emergence is only linear when seed is sown in a similar environment, Hare *et al.*, (1995). A seed lot with 19% seeds infected grown at 6°C produced fewer seedlings than a seed lot with 44% seed infection grown at 8°C, 10°C and 12°C.

There are no standards for *M. nivale* in the EU Cereals Seeds Directive (Anon., 1966) or in any of the UK's Cereal Seeds Marketing Regulations. However, an advisory threshold of five per cent is used to determine whether a seed lot requires treatment to control the disease. If a seed health test provides a result of less than or equal to five percent it is considered safe to sow seed untreated in certain circumstances. Although at present, most winter wheat seed lots in UK are sown with a seed treatment to control *M. nivale*, there has been an increase in the number of crops, particularly farm-saved, sown with untreated seed, Hardwick *et al.,* (2002). This is most likely as a result of growers trying to cut production (variable) costs.

The incidence of seed-borne *M. nivale* fluctuates from year to year (Cockerell & Rennie 1996, Kenyon & Thomas 2001, and Cockerell *et al.*, 2002) with mean seed infection levels ranging from less than 5% to more than 25%. The proportion of samples with greater than five percent *M. nivale* infection in a survey of seed-borne diseases of cereals from 1992 – 1994 ranged from approximately 20% to greater than 80% (Cockerell & Rennie, 1996). During this period levels of infection were lower on Scottish produced wheat than on English produced wheat. In contrast, Hewett 1965 observed that seed produced in the north and west of the UK tended to be more heavily infected with *M. nivale* than seed from the south and east. An analysis of six years data (1994 –1999) by Kenyon and Thomas, 2001 showed a similar variation of seed-borne *M.nivale* levels with samples from the north and south-west of England most likely to be more heavily infected. A survey of Scottish wheat between 1991 and 2000 found no true regional differences in the incidence of *M. nivale* seed infection in the main seed growing areas of Scotland (Cockerell *et al.*, 2002). Mean levels of infection in Scotland during this survey ranged from a low of 3% in 1995 to a high of 42% in 1997. Seed-borne infection is associated with rainfall during flowering (Cockerell *et al.*, 2002, Kenyon & Thomas, 2001) and has no relationship with the infection level on the mother seed.

Data on the relationship between *M. nivale* levels on seed and disease expression already exist (Cockerell, 1995 and Humphreys *et al*, 1995). This project aimed to establish whether 'worst case' outcomes were adequately represented in the existing data and to quantify the relationship between plant population and seed infection using existing and new data. The results would then be used to determine an appropriate seed treatment threshold.

MATERIALS AND METHODS

Seed samples

Seven seed lots of winter wheat with a range of infection levels from 2% to 49% were sourced in autumn 1999 and five seed lots with infection levels ranging from 0% to 35% were sourced in autumn 2000 by the OSTS, Edinburgh. As it was not possible to source a range of *M. nivale* infections' from a single cultivar or site of production, the seed stocks represented a range of cultivars and sites of production, Table 1.

Seed Treatment and Seed Testing

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 Beret Gold® (fludioxonil) 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 (Cockerell & Rennie, 1996); germination (using a giberellic acid pretreatment to break dormancy); tetrazolium; moisture and thousand seed weight (Anon., 1999). Seed test results are given in Appendix 1 for samples used in 1999 and 2000.

Field Experiments

Two field experiments were sown at ADAS High Mowthorpe, Malton, N. Yorks., one in 1999 and the other in 2000 using the samples listed in Table 1. Plot samples were prepared using the thousand seed weight to calculate the quantity of seed required to provide a target seed rate of 450 seeds/m² for each treatment. Plots were 1.5 m wide by 12 m long (18 m²). Plots were drilled using an Oyjord seed drill on 2 November 1999 and 14 November 2000 respectively. Cultivation details and crop inputs are detailed in Appendix II. In both years the experiments were laid out in randomised complete block designs, with three replicates of each treatment.

Table 1 Variety, source and *M. nivale* infection level of seed lots used in the 1999 and 2000 field experiments.

Seed Stock	Variety	Source	% *M. nivale*
1999			
1	Consort	East Lothian	2
2	Riband	East Lothian	9
3	Buchan	East Lothian	12
4	Riband	Aberdeenshire	20
5	Claire	Yorkshire	33
6	Riband	Stirlingshire	34
7	Buchan	Ross-shire	49
2000			
1	Claire	Angus	0
2	Savannah	Clackmannanshire	5
3	Consort	Stirlingshire	11
4	Consort	West Lothian	19
5	Malacca	Mertoun	35

Emergence Counts

Emergence counts were made at Zadoks Growth Stage 12 on 18 January 2000 and on 2 February 2001 respectively. The numbers of plants emerged in 10 x 0.5m rows, selected at random within each plot, were counted.

Yield

Plots were harvested on 5 September 2000 and 28 August 2001 respectively. Yield was calculated in tonnes per hectare at 85% DM.

Statistical analysis

ANOVA was used on emergence and yield data from each experimental year. Data from experiments carried out at SASA in 1991 and 1992 (Cockerell, 1995) was examined together with the ADAS data in an over-trial analysis of the treatment means (over-blocks). It is not believed that there is any significant varietal variability in resistance to *M. nivale* so the possible influence of variety on plant population was ignored.

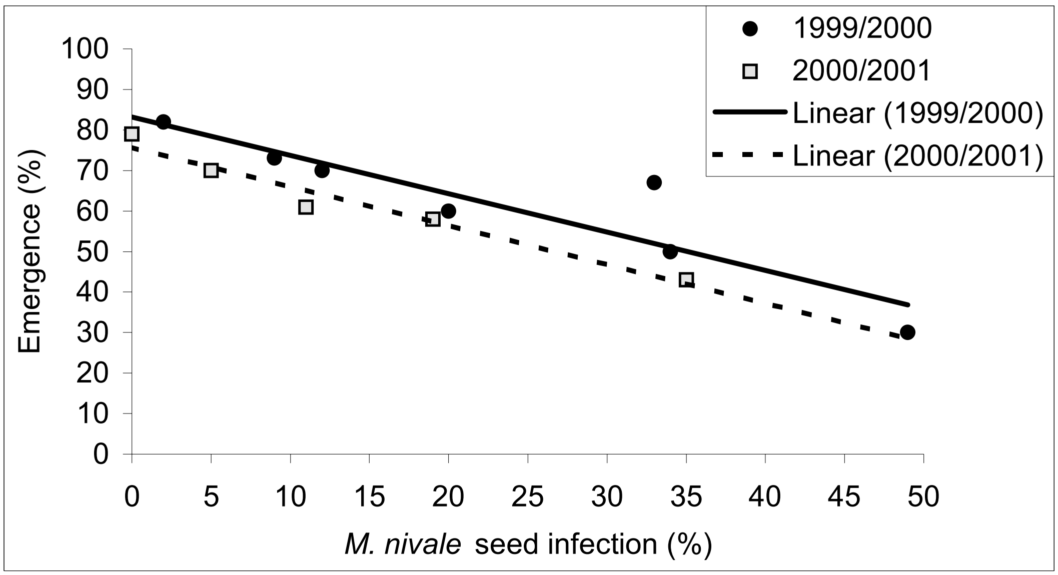

Figure 1 The relationship between *M. nivale* seed infection and emergence in the field.

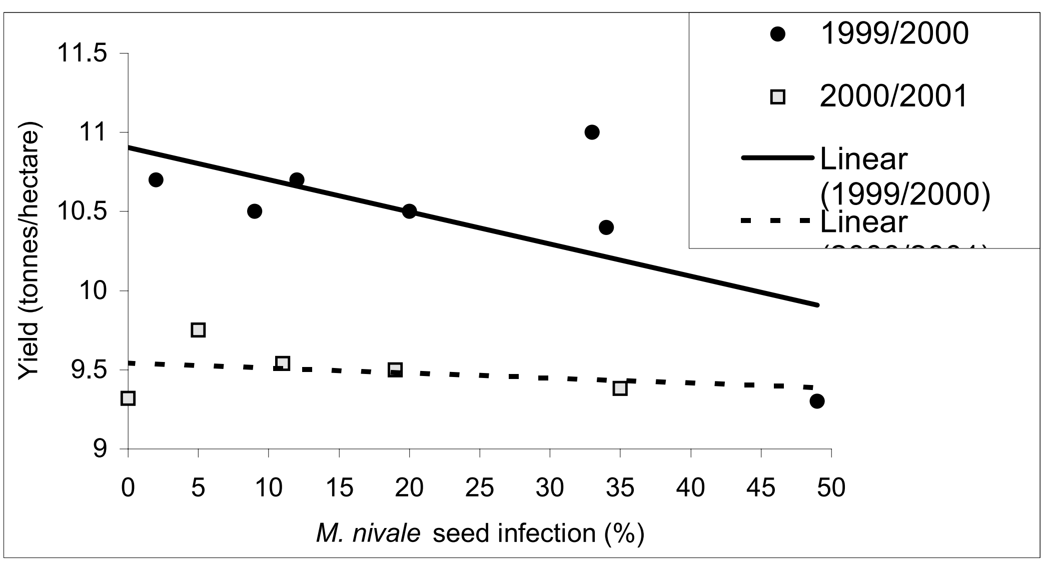

Figure 2 The relationship between *M. nivale* seed infection and yield.

RESULTS

There was a good relationship between the percentage of seed infection and emergence of untreated seed in the field in both years, Figure 1. As the percentage of *M. nivale* on the seed increases, the emergence decreased, Table 2.

Table 2 Slope, intercept and R^2 for both years experiments.

Year	Slope	Intercept	R^2
Emergence			
1999/2000	-0.94	83.2	0.83
2000/2001	-0.96	75.7	0.96
Yield			
1999/2000	-0.02	10.9	0.39
2000/2001	-0.003	9.5	0.07

There was little relationship between yield and percentage seed infection in either year, Figure 2, and Table 2. In 1999/2000, seed treatment increased yield by 0.9t/ha at a seed infection of 49% *M. nivale*, however below this level there was little or no benefit from seed treatment. There was no difference in yield between untreated and treated plots at any seed infection level in 2000/2001.

Quantification of the relationship between plant population and seed infection based on experiments carried out at SASA and ADAS between 1991 and 2001.

The data used to quantify the relationship between plant population and seed infection is summarised in Table 3. The performance of the different seed treatments varied in the SASA experiments. The seed treatment used in the ADAS experiments, fludioxonil, was not present in the SASA experiments of 1991 or 1992. Rappor® (guazatine) was selected from the SASA-tested treatments for over-trial analysis, as this seed treatment performed well in both years. The loss of plants due to sowing untreated seed with *M. nivale* infection was calculated as a percentage of the plant population for treated seed. The data are tabulated in Table 4. The mean plant populations are plotted against seed infection level in Figure 3. Plant loss from untreated seed is plotted against infection level in Figure 4. Linear regressions of plant population on seed infection grouped by experiment were carried out for both untreated and treated seed. In both cases, the analyses indicated that while there was evidence for differences between experiments (p-values<0.001 for both treated and untreated), these differences were reasonably consistent over the range of seed infections tested (p-values for different slopes= 0.81

for untreated and 0.23 for treated). For untreated seed, 3.64 plants per square metre were lost for each 1% increase in seed infection. For treated seed, the loss was less at 0.58 plants per square metre for each 1% increase in seed infection.

It should be noted that the relationship between plant population and seed infection may not be linear. However, linearity seemed a reasonable approximation between 0% and 40% seed infection.

Table 3 Summary of Data sources (Cockerell, 1995 and this paper)

SASA (Cockerell, 1995)

SASA - Gogarbank Farm 1991-2

- Six treatments: untreated control and five fungicide seed treatments
- Eight seed lots: four varieties with seed infection levels from 2% to 61%
- Four complete blocks
- Emergence: seedlings established per square metre
- Sown late October

SASA - Gogarbank Farm 1992-3

- Six treatments: untreated control and five fungicide seed treatments
- Five seed lots: two varieties with seed infection levels from 3% to 77%
- Four complete blocks
- Emergence: seedlings established per square metre
- Sown late October

ADAS 1999/2000 and 2000-2001

ADAS - High Mowthorpe 1999-2000

- Two treatments: untreated and Beret Gold fungicide seed treatments
- Seven seed lots: four varieties with seed infection levels from 2% to 49%
- Three complete blocks
- Emergence: seedlings established per square metre
- Sown start November

ADAS - High Mowthorpe 2000-2001

- Two treatments: untreated and Beret Gold fungicide seed treatments
- Five seed lots: three varieties with seed infection levels from 0% to 35%
- Three complete blocks
- Emergence: seedlings established per square metre
- Sown mid-November

Table 4 Plant populations (plants per square metre) and percentage loss of plants due to sowing untreated seed with *M. nivale* infection as a percentage of the treated seed plant population.

Experiment	Seed Infection (%)	Treated population (number/m^2)	Untreated population (number/m^2)	Loss (%)
SASA 91-92				
	2	280	243	13
	8	273	221	19
	18	271	175	35
	24	273	175	36
	28	259	184	29
	34	266	109	59
	45	252	84	67
	61	261	54	79
SASA 92-93				
	3	422	361	15
	27	392	284	28
	41	348	193	45
	45	383	84	78
	77	375	137	64
ADAS 99-00				
	2	365	368	-1
	9	359	329	8
	12	363	313	14
	20	314	271	14
	33	357	301	16
	34	350	223	36
	49	288	133	54
ADAS 00-01				
	0	366	354	3
	5	361	315	13
	11	337	275	19
	19	351	263	25
	35	378	194	49

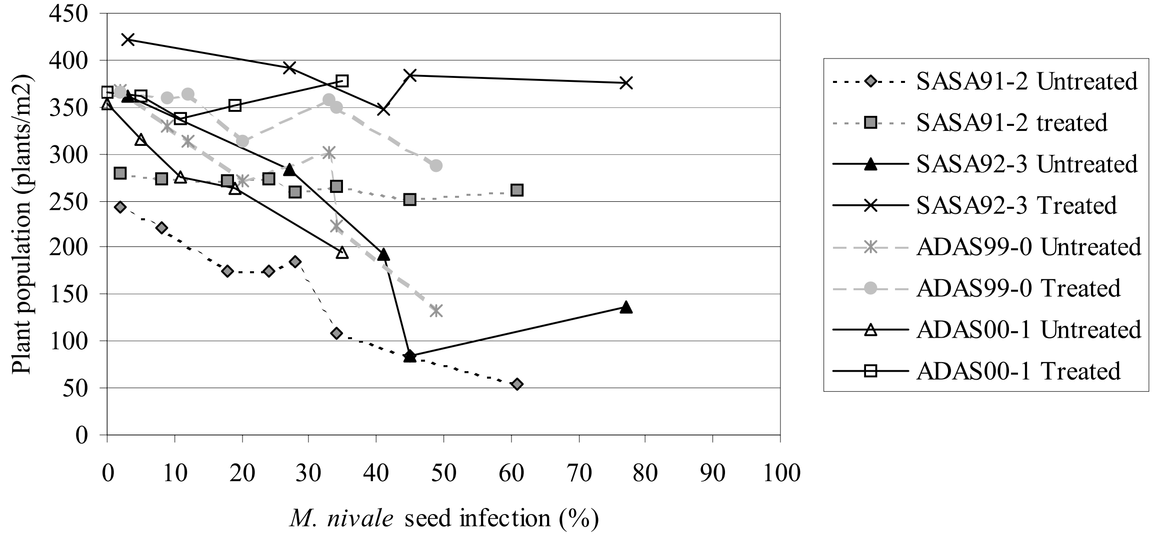

Figure 3 Relationship between plant populations of untreated and treated samples and seed infection.

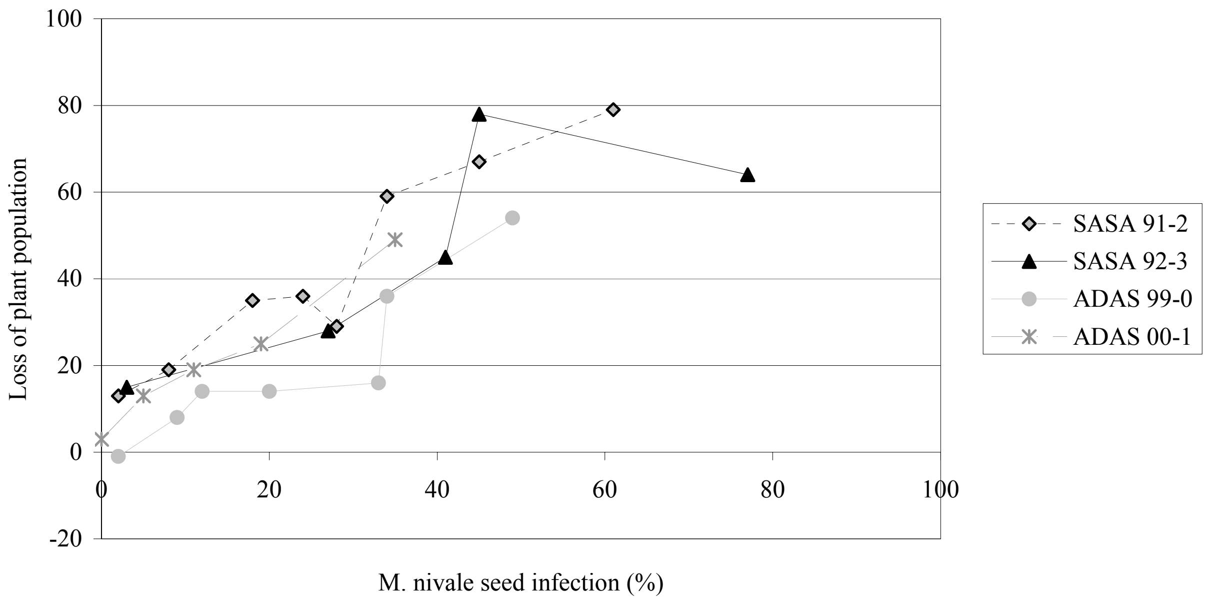

Figure 4 Relationship between plant loss for untreated seed (as a percentage of treated seed plant populations) and seed infection

A linear regression of plant loss against seed infection showed that there was evidence for differences between experiments (p-values<0.001, intercepts: 14.9% SASA 91-2, 7.4% SASA 92-3, -2.4% ADAS 99-0, 7.7% ADAS 00-1). However, these differences were reasonably consistent over the range of seed infections tested (p-value for different slopes= 0.42). The common slope indicated an increase in plant loss of 0.99% for each 1% increase in seed infection.

DISCUSSION

The expression of seed-borne *M. nivale* seedling blight is influenced by drilling date and seedbed conditions (Hare et al., 1995). Where seedbed conditions are favourable for quick germination and establishment the effects of the disease are less noticeable. Where seedbed conditions are conducive to delayed emergence the detrimental effects of the disease are increased. Late drilling at the ADAS site at High Mowthorpe in North Yorkshire was chosen to determine whether 'worst case' outcomes in terms of the relationship between seed infection and emergence were adequately represented in previous experiments (Cockerell, 1995, Humphreys, 1995). The relationship between seed infection level and emergence of untreated seed in both experiments (ADAS 1999/2000 and ADAS 2000/2001) was consistent with the results of studies by Cockerell (1995) and Humphreys (1995).

When comparing the data from various authors on the effect of seed infection on yield, the results are inconsistent. There was no strong relationship between seed infection and yield in either the 1999/2000 or 2000/2001 experiments. In 1999/2000, seed treatment increased yield by 0.9t/ha at a seed infection of 49% *M. nivale*, however below this level there was little or no benefit from seed treatment in either year. Humphreys *et al.*, 1995 observed that variation in *M. nivale* infection levels led to wide differences in establishment, causing significant reductions in grain yield of varieties with high levels of infection. Experiments by Gilchrist & Christie (1996) showed no significant difference in yield when seed lots with 20% and 28% were sown untreated compared to the same lots treated. Where seed lots with *M. nivale* infection greater than 41% infection were sown, there was an increase in yield with treatment.

On the other hand, Burgess *et al.* (1996), found no differences in yield between untreated and treated seed lots with 41% and 11% seed infection. Many winter wheat varieties have considerable potential to tiller to compensate for low seedling numbers and many crops may therefore be able to tolerate significant seedling losses.

Statistical analysis of the data from experiments conducted at Gogarbank Farm, near Edinburgh in 1991 and 1992 and at High Mowthorpe 1999 and 2000 has quantified the relationship between seed infection and the reduction of emergence for untreated seed (compared to treated seed) where conditions are conducive to seedling blight. An increase in seed infection by one per cent produced a reduction in emergence of one per cent. This information can be used to determine a suitable threshold above which there would be a cost benefit in using a seed treatment effective against seedling blight.

The cost of treated winter wheat seed in the UK (autumn 2003) is around £240 per tonne. The minimum cost of seed treatment for *M. nivale* seedling blight is approximately £40 per tonne. The cost of the untreated seed is therefore calculated at £200 per tonne. Figure 5 compares the cost associated with adjusting seed rate to compensate for losses due to *M. nivale* infection (in worst case situations) compared to the cost of seed treatment. The calculations assume a potential germination of 100%.

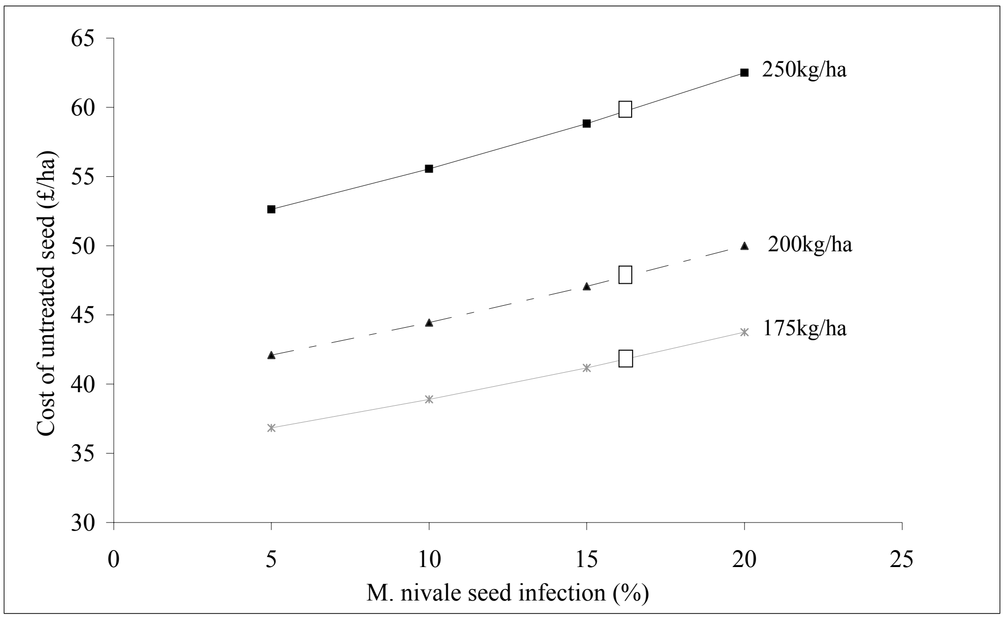

Figure 5 Comparison of costs of untreated seed with treated seed, where untreated seed rate is increased to obtain the same plant population (in worst case situations). Breakeven points shown as white rectangles.

For untreated seed costing £200 per tonne, the breakeven point at which there is no cost benefit from sowing untreated seed is approximately 16.5% *M. nivale* seed infection. The breakeven point is not influenced by seed rate. To sow seed untreated the additional cost of testing for seed-borne pathogens should also be accounted for.

Winter wheat seed should be tested for both *Microdochium* seedling blight and bunt (*Tilletia tritici*) Cockerell *et al* (2004). The cost per hectare associated with testing for seed-borne pathogens is dependant on the size of the seed lot. The maximum recommended seed lot size for seed-borne disease testing is 30 tonnes (Law *et al.*, 2004). The cost of testing per 30 tonnes is approximately £50.00 (2003 prices). The costs per hectare for a range of seed lot sizes and seed rates are given in Table 6. Including seed testing costs for untreated seed produced at £200 per tonne would reduce the breakeven point to 15% *M. nivale* infection where ten tonnes of seed are produced and approximately 16% for 30 tonnes.

Seed health testing results, like all tests, have an element of uncertainty around the result given. Tables produced by Miles, 1963 have proven to be a good measure of uncertainty for the agar plate test used to determine the level of *M. nivale* in these experiments. Table 7 details the confidence intervals at 95% and 99% confidence for test results from 10% - 18%.

Table 6 The cost of seed testing per hectare (£) for a range of seed lot sizes and seed rates.

Seed rate / Seed lot size	30 tonnes	20 tonnes	10 tonnes
250 kg/ha	0.42	0.63	1.25
200 kg/ha	0.33	0.50	1.00
175 kg/ha	0.29	0.44	0.88

Table 7 Confidence intervals associated with *M. nivale* agar plate test results.

% *M. nivale* (test result)	Confidence intervals	
	95%	99%
10	6-15	6-17
12	8-17	9-17
14	10-20	9-22
16	11-22	10-24
18	13-24	12-26

To ensure that the level of *M. nivale* is not greater than the breakeven point at which seed treatment would provide a benefit, the level of infection at which seed could be sown untreated is 10% at 95% confidence limits.

Prediction of *Microdochium* seedling blight is difficult as environmental conditions at and after sowing will determine the extent of disease expression. Evidence suggests that a small reduction in the number of established plants even in worst case situations may be compensated for by increased tillering. Where seeding rates of over 350 seeds per m^2 are the target, and the seed infection level is 10% or less, increasing the sowing rate because of *M. nivale* is not advised. Where growers are using very low seed rates seed treatment is recommended.

A 10 per cent threshold would allow between 9% and 94% of winter wheat seed lots to be sown untreated in respect of *M. nivale*, in any one year, in Scotland and between 35% and 99% in England and Wales, Figure 6.

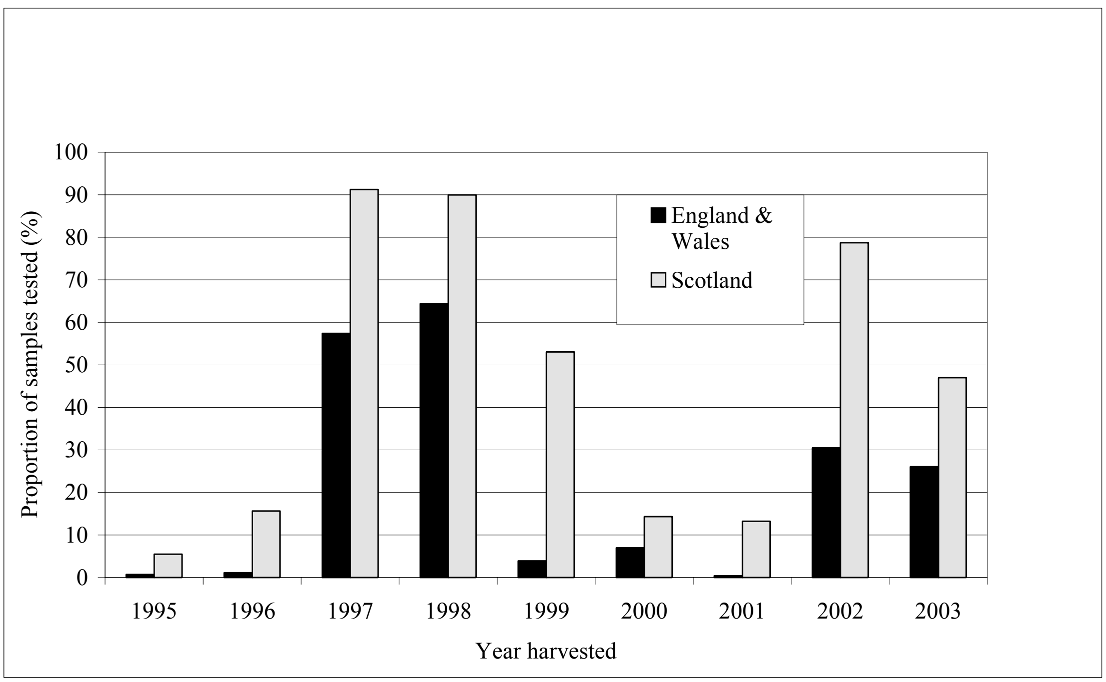

Figure 6 The proportion of samples greater than 10% *M. nivale* seed infection tested in Scotland and England and Wales.

CONCLUSIONS

- *M. nivale* can cause reduced emergence in winter wheat crops. Winter wheat seed must be tested for *M. nivale* before sowing seed untreated.

- The relationship between *M. nivale* seed infection and emergence has been quantified in worst case situations. An increase in seed infection of one per cent produces an increase in plant loss of one per cent.

- A ten per cent threshold has been calculated above which the benefits of seed treatment would be cost effective where late sowing or where seedbed conditions will delay emergence.

- A ten per cent threshold would mean that between 9% and 94% of winter wheat seed lots could be sown untreated in respect of *M. nivale*, in any one year, in Scotland and between 35% and 99% in England and Wales.

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Table 1 Seed Testing Results - Autumn 1999 seed stocks

			Seed Testing Results				
Seed stock	Treatment	OSTS Reference	*M. nivale* %	Germination %	Tz % viable	% Moisture	TSW g
1	None	9/1294 Consort	2	98	99	14.4	51.1
2	None	9/1479 Riband	9	96	95	14.6	40.9
3	None	9/1480 Buchan	12	96	97	13.0	51.6
4	None	9/1296 Riband	20	87	96	15.5	45.5
5	None	9/1297 Claire	33	97	98	16.0	47.1
6	None	9/1610 Riband	34	92	98	15.9	46.4
7	None	9/1435 Buchan	49	71	97	17.5	46.0
8	Beret Gold	9/1640 Consort	2	97			
9	Beret Gold	9/1639 Riband	9	97			
10	Beret Gold	9/1641 Buchan	12	99			
11	Beret Gold	9/1643 Riband	20	93			
12	Beret Gold	9/1642 Claire	33	97			
13	Beret Gold	9/1638 Riband	34	95			
14	Beret Gold	9/1629 Buchan	49	92			

Table 2 Seed Testing Results - Autumn 2000 seed stocks

			Seed Testing Results				
Seed stock	Treatment	OSTS Reference	*M. nivale* %	Germination %	Tz % viable	Moisture %	TSW g
1	None	00/1889 Claire	Nil	98	97	13.8	41.6
2	None	00/1893 Savannah	3	90	89	13.7	59.8
3	None	00/1890 Consort	8	89	87*	13.4	49.5
4	None	00/1891 Consort	24	94	98	13.6	51.6
5	None	00/1892 Malacca	31	93	99	13.1	44.0
6	Beret Gold	00/1902 Claire	Nil	97	-	-	42.5
7	Beret Gold	00/1905 Savannah	3	92	-	-	58.8
8	Beret Gold	00/1903 Consort	8	95	-	-	48.5
9	Beret Gold	00/1901 Consort	24	96	-	-	51.5
10	Beret Gold	00/1904 Malacca	31	97	-	-	43.3

* Evidence of heat damage

Appendix II

Site Details 1999/2000 and 2000/2001 ADAS Experiments

Site:	ADAS High Mowthorpe 1999/2000		
Field Name:	Elbow North		
Soil:	Shallow silt clay loam overlying chalk.		
		ADAS Index	
Soil Analysis	P	2	
	K	2	
	Mg	1	
	Organic matter %	4.3	
	pH	7.9	
Drainage	Good		
Cultivations:	Ploughed		
	Drilled	2 November 1999	
	Rolled	3 November 1999	
Date of planting:	2 November 2000		
Cultivar:	As per Materials & Methods		
Seed rate (kg/ha)	As per Materials & Methods		
	Application date	Product	Rate
Herbicides:	27 Jan 00	Stomp +	2 l/ha +
		IPU	2 l/ha
PGR's:	28 April 00	Cycocel 720	2.3 l/ha
Fungicides:	10 May 00	Amistar +	0.6 l/ha
		Impact Excel	
	5 June 00	Twist +	1.0 l/ha +
		Caramba	0.5 l/ha
Insecticides:	None		
Molluscicides:	27 Nov 99	Draza	5.5 kg/ha
		<u>Date</u>	<u>Nutrient rate</u>
Fertiliser inputs:	N	8 Mar 00	40 kg/ha N (Extram 125 kg/ha)
		17 Apr 00	163 kg/ha n (Extram 472 kg/ha)
	P	Nil	
	K	Nil	

Site Details 1999/2000 and 2000/2001 ADAS Experiments

Site:	ADAS High Mowthorpe 2000/2001		
Field Name:	Old Type (North East)		
Soil:	Shallow silt clay loam overlying chalk.		
		ADAS Index	
Soil Analysis	P	2	
(1996)	K	1	
	Mg	1	
	Organic matter %	4.05	
	pH	8.0	
Drainage	Good		
Cultivations:	Ploughed	18 October 2000	
Date of planting:	14 November 2000		
Cultivar:	As per Materials & Methods		
Seed rate (kg/ha)	As per Materials & Methods		
	Application date	Product	Rate
Herbicides:	15 Feb 01	Encore	4 /ha
	30 Apr 01	Eagle	30g/ha
PGR's:	30 April 01	Belcocel 720	2.33 l/ha
Fungicides:	20 May 01	Opus	0.6 l/ha
	20 May 01	Twist	0.8 l/ha
	6 June 01	Landmark	0.53 l/ha
	4 July 01	Folicur	0.27 l/ha
Insecticides:	27 Feb 01	Dimethoate	1.7 l/ha
	27 Feb 01	Dursban 5	1.5 l/ha
Molluscicides:	27 Nov 00	Draza	5.5 kg/ha
		<u>Date</u>	<u>Nutrient rate</u>
Fertiliser inputs:	N	13 Mar 01	44 kg/ha N (Nitram)
		4 May 01	189 kg/ha n (Nitram)
	P	Nil	
	K	Nil	

Data: 1999/2000 Experiment

Table 1 Plants/m² (18 January 2000)

% *M. nivale*	Untreated	+ Beret Gold	**Mean**
2	367	365	366
9	329	359	344
12	312	362	338
20	270	313	292
33	300	356	329
34	222	350	286
49	132	287	210
Mean	268	342	

	% *M. nivale*	Seed treatment	Interaction
F.pr (26 d.f.)	<0.001	<0.001	<0.001
SE/Mean	8.6	4.6	12.2
SED	12.2	6.5	17.2
CV%			6.8

Table 2. Yield (t/ha @ 85% DM).

% *M. nivale*	Untreated	+ Beret Gold	Mean
2	10.7	10.8	10.7
9	10.5	10.9	10.7
12	10.7	10.7	10.7
20	10.5	10.9	10.7
33	11.0	11.0	11.0
34	10.4	10.9	10.6
49	9.3	10.2	9.7
Mean	10.4	10.8	

	% *M. nivale*	Seed treatment	Interaction
F.pr (18 d.f.)	0.39	0.03	0.35
SE/Mean	0.069	0.109	0.155
SED	0.098	0.155	0.219
CV%			2.8

Data: 2000/2001 Experiment

Table 3 Plants/m² 2 February 2001

% *M. nivale*	Untreated	+ Beret Gold	**Mean**
0	354	366	360
5	315	361	338
11	275	337	306
19	263	351	307
35	194	378	286
Mean	280	359	

	% *M. nivale*	Seed treatment	Interaction
F.pr (18 d.f.)	<0.001	<0.001	<0.001
SE/Mean	6.82	10.78	15.25
SED	9.64	15.25	221.57
CV%			8.3

Table 4. Yield (t/ha @ 85% DM).

% *M. nivale*	Untreated	+ Beret Gold	**Mean**
0	9.32	9.55	9.43
5	9.75	9.79	9.77
11	9.54	9.19	9.37
19	9.50	9.44	9.47
35	9.38	9.09	9.23
Mean	9.50	9.41	

	% *M. nivale*	Seed treatment	Interaction
F.pr (18 d.f.)	0.39	0.03	0.35
SE/Mean	0.069	0.109	0.155
SED	0.098	0.155	0.219
CV%			2.8