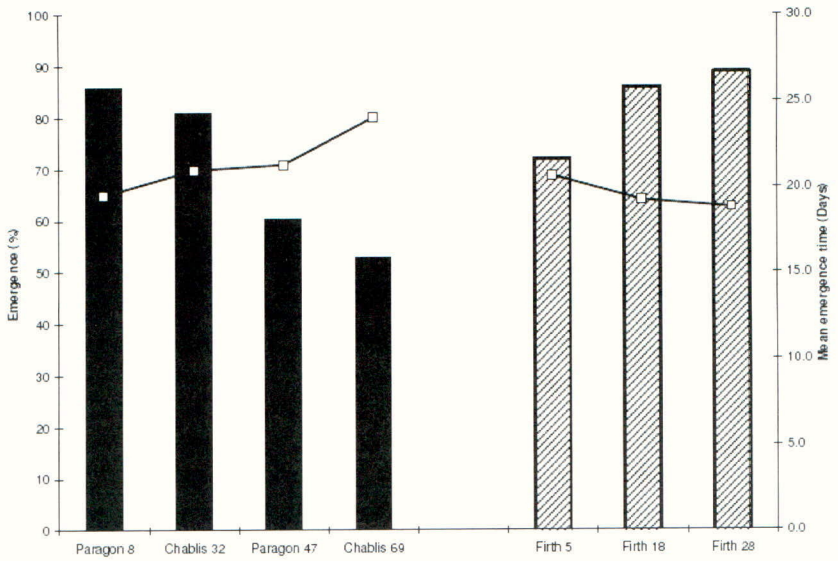

Figure 1 Maximum emergence (columns) and mean emergence time (line) for wheat and oat seed lots infected with *Microdochium nivale* sown in pots with compost held at 75% field capacity

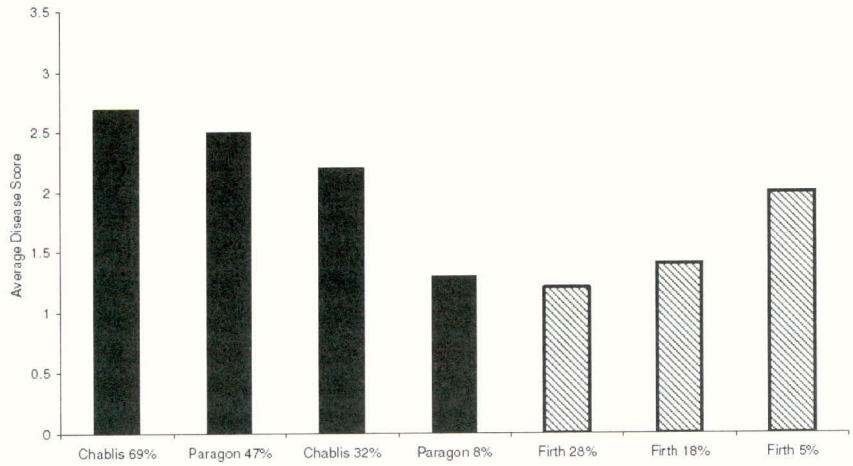

Figure 2 Average disease scores for wheat and oat seed lots infected with *Microdochium nivale* sown in pots with compost held at 75% field capacity

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.

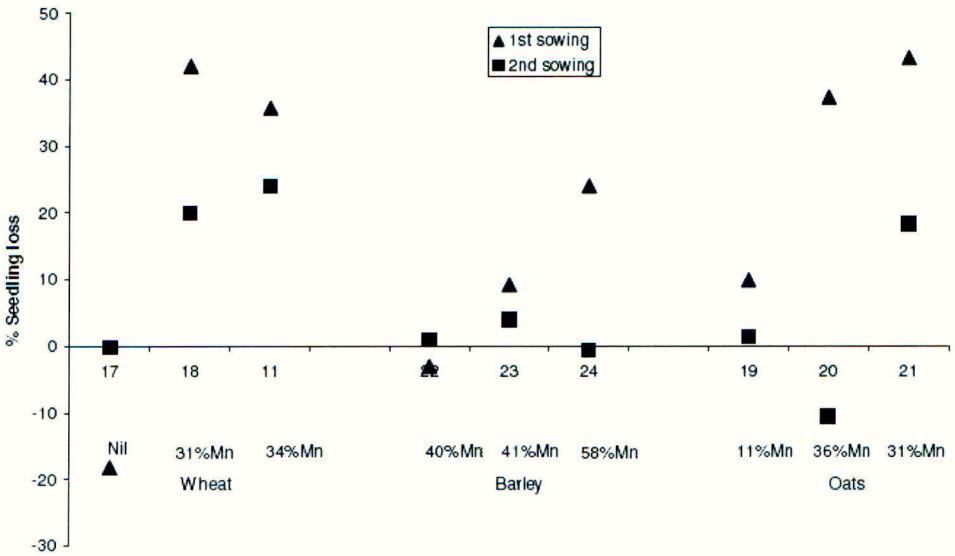

Figure 3 Mean seedling loss for spring wheat, spring barley and spring oats sown 4 April 2008 and 22 April 2008 at Gogarbank Farm, Edinburgh

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.

References

Anon. (2009) Chapter 5. *International Rules for Seed Testing*. International Seed Testing Association, Basserdorf, Switzerland

Cockerell V; Clark WS; Roberts AMI (2004) The effect of *Microdochium nivale* on the quality of winter wheat seed in the UK. Technical Paper No. 4 in *Cereal Seed Health and Seed Treatment Strategies: Exploiting New Seed Testing Technology to Optimise Seed Health Decisions for Wheat.* Home-Grown Cereals Authority Project Report No. 340. Home Grown Cereals Authority, London.

Richardson MJ; Whittle AM; Jacks M (1976) Yield–loss relationships in cereals. *Plant Pathology* **25**, 21–30.

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

M McNeil and V Cockerell

SASA, Roddinglaw Road, Edinburgh EH12 9FJ, UK

Email: Marian.McNeil@sasa.gsi.gov.uk

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

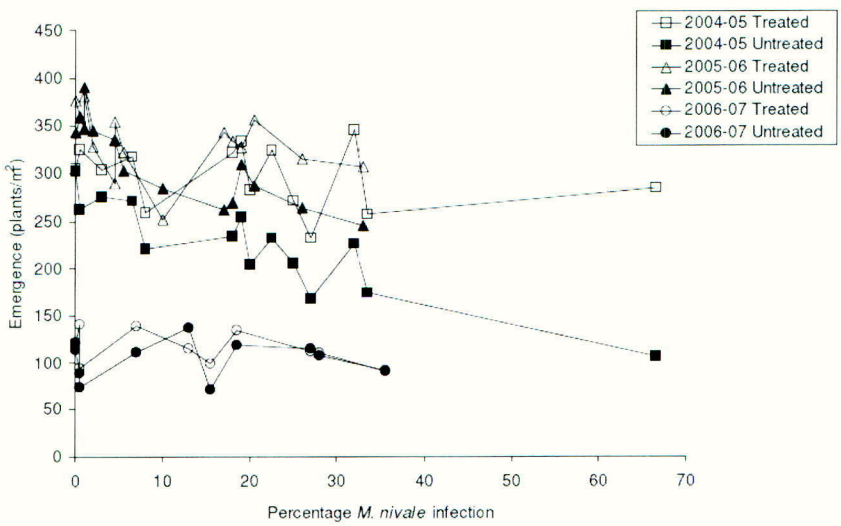

Figure 1 Mean untreated and treated emergence counts (plants/m^2)

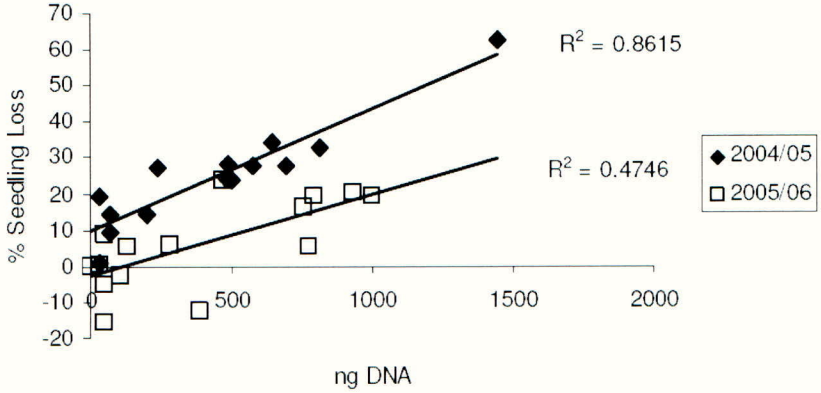

Figure 2 Percentage plant loss 2004/05 and 2005/06 compared with ng DNA *Microdochium nivale*

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.

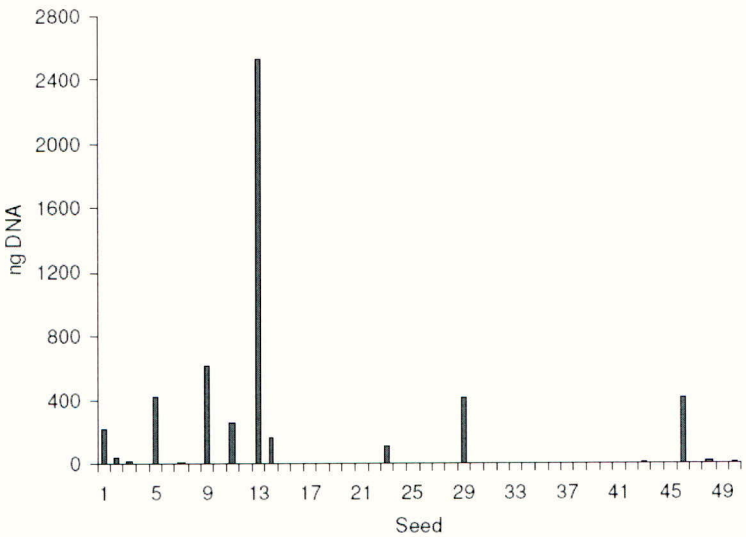

Figure 3 Single seed extracts showing the different magnitudes of inoculum loading on individual seeds

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

Anon. (2009) Chapters 5, 6, 7 and 11, *International Rules for Seed Testing. International Seed Testing Association, Basserdorf, Switzerland.*

Cockerell V; Paveley ND; Clark WS; Thomas JE; Anthony S; McEwan M; Bates J; Roberts AMI; Law J; Kenyon DM; Mulholland V (2004) 'Cereal seed health and seed treatment strategies: exploiting new seed testing technology to optimise seed health decisions for wheat', HGCA Project Report No. 340. HGCA, London.

Edwards SG; Pirgozliev SR; Hare MC; Jenkinson P (2001) Quantification of trichothecene-producing *Fusarium* species in harvested grain by competitive PCR to determine efficacies of fungicides against *Fusarium* head-blight of winter wheat. *Applied and Environmental Microbiology*, **67**, 1575–1580.

Seed testing preventing the introduction of quarantine pathogens

K J D Hughes, V L Barton, J Elphinstone and R Mumford

Plant Health Group, Central Science Laboratory, Sand Hutton, York, North Yorkshire YO41 1LZ, UK

Email: k.hughes@csl.gov.uk

Seed-borne plant pathogens can be easily moved around the world into new environments, which, if favourable to the pathogen, can lead to poor plant establishment as well as a reduction in the yield and quality of the crop (direct costs). Trade restrictions may be imposed on the affected country or region if the pathogen concerned is listed as a quarantine pest by other importing countries (reaction costs). Affected countries may have to implement stringent and costly control strategies to deal with outbreaks in order to regain lost export markets. As such, many countries require seed to be certified free from these pathogens as part of their phytosanitary import requirements. Several organisations and initiatives publish protocols to diagnose seed-borne pathogens, including the International Seed Testing Association (ISTA) and International Seed Health Initiative (ISHI). Other protocols are also published in handbooks (e.g. Albrechtsen, 2006; Mathur & Kongsdal, 2003; Saettler *et al.*, 1989) and scientific journals such as *Seed Science & Technology*. Unfortunately, many of these rely on destructively testing large numbers of seed, some of which, such as tomato and tree seed, can be extremely valuable. This can make some exports untenable on the grounds of cost and/or the availability of test seed.

Increasingly, many modern diagnostic technologies such as ELISA and real-time PCR are being used alongside traditional methods, including isolation and blotter tests, to aid pathogen diagnosis and reduce the spread of plant disease around the world. Non-destructive methods are also employed when available. Examples of EU quarantine seed-borne pathogens which are routinely tested for using modern diagnostic technologies include *Tilletia indica*, *Pantoea stewartii* and *Pepino mosaic virus*.

Tilletia indica causes the fungal disease of wheat and triticale known as Karnal bunt. Infected seeds may be partially bunted, containing many thousands of teliospores, or they may carry only a few teliospores on their surface. These teliospores have been shown to remain viable under European field conditions for at least 3 years. Further, it has been estimated that if *T. indica* occurs as a small (1000 ha) or large (50,000 ha) outbreak in the UK, it could cost the UK within the first year between 1.7 and 17.8 million Euro in direct, reaction and control costs (Sansford *et al.*, 2006). In 2004 the European Plant Protection Organization (EPPO) published its standard for diagnosis of *T. indica*, which has been adopted as the EU standard by many plant health laboratories. This involves sieving samples for teliospores, followed by their morphological and molecular assessment if required (Anon., 2004).

Pantoea stewartii, formerly known as *Erwinia stewartii*, causes bacterial wilt of maize, which is thought to be indigenous to America (Anon., 2006). It is thought that this pathogen is brought to new areas by seed and, once established, is spread by insect vectors (Anon., 1997). Various methods exist to diagnose infection in seed and frequently these are used in combination, as outlined in the EPPO standard for *P. stewartii* (Anon., 2006).

Pepino mosaic virus can infect a number of solanaceous hosts including pepino, tomato and potato. In tomato, spread to new areas is thought to be via infected seeds and seedlings and, once established in a crop, the virus is highly contagious and can lead to the downgrading of fruit, costing at least £16 m^{-2} based on 2005 prices (Spence *et al.*, 2006). Seed can be tested by a number of methods, including ELISA and real-time PCR. Traditionally this has been performed by destructive testing, but a recent industry-funded project has shown that non-destructive methods for diagnosis can also be employed (Mumford, 2006).

Ideally, as new methods are developed these should be made available to trade and government laboratories through peer-reviewed publications. Further, they should also be independently assessed through 'blind-testing' schemes to confirm their validity and to identify if they have advantages over established tests. However, the organisation and cost of running such schemes can be enormous, and the take-up of any new methods, particularly when involving technologies such as real-time PCR, may require high set-up and running costs. If these issues can be addressed, this will no doubt promote the uniformity and take-up of such methods and as a consequence reduce the spread and introduction of alien pathogens. The ability to test some seed lots using non-destructive methods, as highlighted by Mumford (2006), is also likely to bring many advantages to trade and governments alike.

References

Anon. (1997) EPPO datasheet on *Pantoea stewartii* subsp. *stewartii*. *Quarantine Pests for Europe*, CABI, UK

Anon (2004) Diagnostic protocol for regulated pests: *Tilletia indica*. *EPPO Bulletin*, **34**, 219–227.

Anon (2006) Diagnostic protocol for regulated pests: *Pantoea stewartii* subsp. *stewartii*. *EPPO Bulletin*, **36**, 111–115.

Albrechtsen, S E (2006) *Testing Methods for Seed-Transmitted Viruses: Principals and Protocols*. CABI Publishing, Wallingford, UK

Mathur S B; Kongsdal O (2003) *Common Laboratory Seed Health Testing Methods for Detecting Fungi*. International Seed Testing Association, Zurich, Switzerland.

Mumford R (2006) 'Wash and Grow'; the development of non-destructive tomato seed testing. HDC final report December 2006. Horticultural Development Council, East Malling, Kent, UK.

Saettler A W; Schaad N W; Roth D A (1989) *Detection of Bacteria in Seed and other Planting Material*. The American Phytopathological Society, St Paul, USA.

Sansford C; Baker R; Brennan J; Ewert F; Gioli B; Inman A; Kelly P; Kinsella A; Leth V; Magnus H; Miglietta F; Murray G; Peterson G; Porta-Puglia A; Porter J R; Rafoss T; Riccioni L; Thorne F; Valvassori M (2006) Pest Risk Analysis for *Tilletia indica* for the European Union. (2006). http://karnalpublic.pestrisk.net/

Spence N J; Basham J; Mumford R A; Hayman G; Edondson R; Jones D R (2006) Effect of Pepino mosaic virus on the yield and quality of glasshouse-grown tomatoes in the UK. *Plant Pathology*, **55**, 595–606.

A laboratory test to evaluate the selectivity of seed treatments in cereals

B Mériaux[1] and C Doucet[2]

[1]Fédération Nationale des Agriculteurs-Multiplicateurs de Semences, Impasse du verger, 49800 Brain sur l'Authion, France; [2]LABOSEM, Impasse du verger, 49800 Brain sur l'Authion, FRANCE

Email: benoit.meriaux@fnams.fr

Summary

Seed treatments are applied to a large proportion of certified seeds of cereals in France. Seed companies need information on the effectiveness and selectivity of seed-treatment products. Here, a laboratory method has been developed to evaluate the selectivity of seed treatments. This method can be used all year round, and is cheap compared with field trials. It requires the choice of susceptible seed lots and a germination test in suboptimal conditions (5°C for 4 weeks in sand at holding capacity). Selectivity is evaluated from the number of normal seeds in the test which result in dead seeds or in abnormal seedlings with short roots. This method is now used by seed-treatment producers in order to evaluate the selectivity of seed treatment formulations.

Introduction

Wheat (*Triticum aestivum*) is the most important seed production in France, with about 400,000 tons produced per year. Seed treatments are widely used, and many seed-treatment products are commercialised in France. Seed companies are interested in the effectiveness of seed treatments for protecting the seed and the emerging crop. But they also need to sell treated seeds with good vigour. Phytotoxic effects of seed treatments include poor germination, delayed establishment, and lack of first tiller. They may result from the selectivity of the seed treatment, susceptibility of the seed lot (about 5% seed lot tested), and suboptimal pedoclimatic conditions during germination and emergence. As field conditions favourable to phytotoxicity are difficult to anticipate, evaluation of selectivity in field trials is difficult to develop, and only a few results are workable. So to inform French seed companies about the selectivity of seed treatments commercialised in France, our laboratory developed, with the financial support of the Groupement National Interprofessionnel des Semences (GNIS), a specific method to evaluate the selectivity of seed treatments.

Materials and methods

Our method associates the choice of specific seed lots and a germination test in suboptimal conditions. Two seed lots, used in this study (L1 and L2), were chosen from several wheat seed lots harvested in different part of France in 2003. The standard germination of untreated seeds was above 95% (96 and 95.5%, respectively) and no fungal pathogen was detected by

sanitary analysis. Seed lot L1 was susceptible to seed treatment and seed lot L2 was only slightly susceptible.

Seeds were treated with four different treatments (TS1 to TS4) plus a reference seed treatment (Ref). The reference seed treatment presents a low selectivity. The registration of the four treatments was in progress when we initiated this study in 2004. The seed treatments were applied using a small-batch seed treater HEGE 11 by the slurry method at 1 l/q. After treatment, seeds were dried for 24 h at ambient temperature.

Two germination tests were conducted at LABOSEM with treated and untreated seeds. The standard germination test (7 days at 20°C in sand) according to International Seed testing Association (ISTA) recommendations was performed on 200 seeds. The other test is specific to evaluate the selectivity of seed treatment. This test is performed in sand at holding capacity at cold temperature (5°C) over 4 weeks. At the end of the 4 weeks, normal and abnormal seedlings and dead seeds were counted and typology of abnormal seedlings detailed. The test was performed on 200 seeds.

Statistical analysis was performed using the tolerance tables produced by ISTA. Those tables are based on the mean comparison of 200 seed results.

Results

In optimal conditions, germinations ranged from 90.5 to 97% (Figure 1). Seed lot L1 showed no significant difference of standard germination. The reference product applied on seed lot L2 induced a slight decrease in standard germination. The standard germination test cannot be used in order to evaluate selectivity of seed treatments.

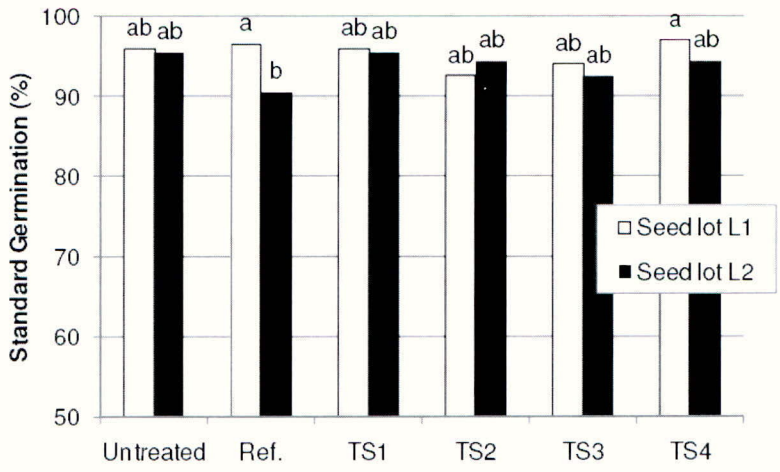

Figure 1 Standard germination (7 days at 20°C) of treated seeds of two seed lots. Different letters represent different germination results.

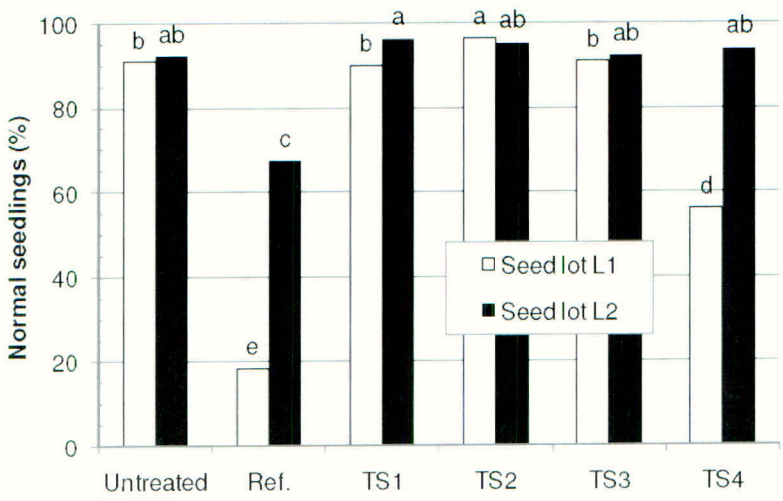

Figure 2 Normal seedlings after germination testing in suboptimal conditions (4 weeks at 5°C in sand at holding capacity). Different letters represent different germination results.

In suboptimal conditions, germination ranged from 18.5 to 96.5% (Figure 2). This test induced large differences in germination between seed lots and between seed-treatment products. Seed lot L1, treated with the reference or TS4, showed lower germination (18.5 and 56%, respectively) than ST1, ST2 and ST3. For the latter three treatments, germination was as good as for untreated seeds. Seed lot L2 showed a better overall germination. For this seed lot, only the seeds treated with the reference showed poor germination (67.5%).

The main cause of poor germination was the presence of abnormal seedlings with very short roots (less than 3 cm) compared with normal seedlings (about 6 cm long). This category of abnormal seedlings was the most frequent, and the treatments with the poorest germination were those with the highest percentage of abnormal seedlings with short roots.

Conclusion

A germination test in suboptimal conditions (cold temperature and sand at holding capacity) was developed at LABOSEM in order to evaluate the selectivity of seed-treatment products. Associated with a preliminary choice of seed lots, it gives unbiased results on the behaviour of new seed-treatment products. This test is more useful than field trials because discrimination of seed treatment selectivity is possible; the test can be used at any time during the year, and gives a result in a short time (4 weeks). This test was initially developed for the seed companies, but now is also used by seed-treatment producers in developing new formulations.

The effect of substrate when testing standard germination of treated maize seed

B Hamman and G Koning

Syngenta Crop Protection, Seed Care Institute, Schaffhauserstrasse, CH-4332, Stein, Switzerland

Email: brigitte.hamman@syngenta.com

Introduction

The rules of the International *Seed Testing* Association (ISTA, 2009) and of the American Association of Official Seed Analysts (AOSA, 1998) describe methods for standard germination (SG) testing of seed. Within the rules, testing conditions are specified for a wide range of species. For most species there are options with regard to the choice of substrate used during the test. These options have been validated internationally to show equivalent germination performance for seedlots under evaluation.

Over the past few years, reports of poor germination performance of maize seed in an SG test after the application of a systemic insecticide seed treatment were at odds with the successful field performance of those same seedlots. These reports came mainly from European countries in which sand is a popular substrate used in the SG test.

The objective of this small study was to compare the germination performance of a number of maize seedlots in an SG test after application with an insecticide using a number of different substrates.

Materials and methods

In two separate experiments, various maize (*Zea mays* L.) seedlots with germination above 90% were treated with CRUISER® (thiamethoxam, 0.63 mg/seed), then reassessed for germination using sand, rolled paper towels and compost (experiment 1 only). All substrates used are permitted for SG testing in the ISTA rules. Experiment 1 consisted of nine seedlots of the same variety and production year; experiment 2 consisted of 35 seedlots from the same production year, but a range of varieties. The seedlots were grouped according to vigour (high, medium and low) based on germination performance of untreated seed in a rolled-towel cold test (Hampton & Tekrony, 1995).

Results and discussion

In experiment 1, it made no difference to the final germination of untreated seed which substrate was used (Figure 1). However, when the same seedlots were tested after treatment with thiamethoxam, the substrate used clearly influenced final germination – when tested in sand, mean germination values were lower than when tested in either compost or in rolled-paper towels. Since only one variety was involved, the experiment was repeated with a group of seedlots that ranged in vigour level and represented a number of different varieties.