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Effects of imidacloprid cereal seed treatment against wireworms and slugs

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ABSTRACT

Imidacloprid as a cereal seed treatment gave good protection against *Agriotes* spp. (wireworm) in the UK. It was equivalent to gamma HCH both in improving crop emergence and reducing *Agriotes* damaged plants.

Slug feeding on germinating wheat was reduced by an average of 68% where seed were treated with imidacloprid. In field trials, these effects translated into improved crop stand but slug grazing on emerged foliage was not much reduced.

INTRODUCTION

Imidacloprid as a winter cereal seed treatment was introduced into the UK in 1998, where it is available in fungicide co-formulations as 'Raxil Secur' for barley, 'Sibutol Secur' for wheat and oats, and as 'Baytan Secur' for wheat, barley and oats. The original development of the a.i. in cereals investigated efficacy against BYDV transmitting aphids (Schmeer *et al.*, 1990). However, imidacloprid has a broader spectrum of insecticidal activity, and further work is reported here on control of *Agriotes* spp. (wireworm). This information is particularly timely for the UK as the former standard wireworm seed treatment, gamma HCH, was withdrawn in 1999.

During the development of these more traditional insecticidal uses, a further beneficial aspect of treating seed with imidacloprid was observed. In a few trials, hints of reduced damage by slugs (usually *Deroceras reticulatum)* were recorded. This effect was confirmed in slightly artificial field tests which are reported here. The relevance to normal usage is also demonstrated, as reported below.

MATERIALS AND METHODS

Trials against *Agriotes* spp.

A number of replicated field trials were done using imidacloprid seed treatment on winter and spring cereals between 1992 and 1999 in *Agriotes* susceptible locations. In the 13 trials reported here, *Agriotes* attack was adequate to generate useful information.

Imidacloprid was applied as a 350 FS and gamma HCH as a 300 FS. A fungicide seed treatment was also co-applied.

Trials were of randomised block design with plots typically 1.5 m x 10 m sown at a seed rate of 180 kg/ha.

Assessments of crop stand were carried out where *Agriotes* attack occurred soon after sowing. In other sites, where damage was recorded later on, counts of *Agriotes* damaged plants were made.

Agriotes presence at sites was confirmed by burying potato tubers as bait, unearthing the tubers, cutting them open and identifying the *Agriotes* which had been attracted to feed.

Trials against slugs

Six randomised block field tests in 1990-91 exposed 5 g lots of treated (imidacloprid 350 FS) wheat seed to slug attack. The seed were placed on the soil surface in fields with high slug activity and covered to reduce access by fauna other than slugs. The seed were recovered and counts of slug hollowed grain were recorded.

In autumn 1999 winter wheat was drilled in 6 randomised block field trials at slug infested sites. Plots were 3 m x 12 m and seed (cv. Consort) drilled at 160 kg/ha. The seed was treated either with 'Sibutol' (bitertanol 375 + fuberidazole 23 g/litre FS) at 150 ml/100 kg seed or the equivalent imidacloprid co-formulation (bitertanol 140 + fuberidazole 8.6 + imidacloprid 87.5 g/litre FS) at 400 ml/100 kg seed. These treatments were either left untreated or treated with 'Rivet' slug bait (methiocarb 3%) at 5 kg/ha. The slug baits were applied in two different ways for each seed treatment. Either an admix with the seed was used, so that the bait was drilled into the soil along with the seed, or a topical broadcast application was made immediately after drilling.

Efficacy was measured by counts of emerged plants and counts of emerged plants showing slug damage.

RESULTS

Agriotes spp.

Table 1 shows the crop stand results from 11 trials. Imidacloprid at 35 g/100 kg seed performed similarly to, and usually a little better than, gamma HCH at 24 g/100 kg seed.

Table 1 (1 of 2). Relative crop stand results from *Agriotes* trials.

Trial	WR-04-92	AD-21-97	AD-81-97	AD-84-97	AE-22-97	AD-82-97	BJ-21-97
Crop	W wheat	W wheat	S barley	S barley	W wheat	S wheat	W wheat
Location	Herefords.	Somerset	Somerset	Somerset	Glos.	Glos.	W Mids.
Drilled	15.11.91	01.10.96	03.04.97	18.04.97	14.10.96	01.04.97	11.10.96
Assessed	22.01.92	22.11.96	07.05.97	27.05.97	05.11.96	13.05.97	25.11.96
Crop GS	14	13	13	14	12	13	13
Insecticide seed treatment g a.i./100 kg seed							
Untreated	100	100	100	100	100	100	100
(Plants/m² in untreated)	(272)	(264)	(139)	(221)	(219)	(111)	(234)
Gamma HCH 24 g	103	110	137	110	111	140	129
Imidacloprid 35 g	118	107	161	115	130	144	135

Table 1 (2 of 2).

Trial	EA-23-97	EA-83-97	SW-09-97	SR-15-99	Mean	(Range)
Crop	W wheat	S barley	W wheat	W wheat		
Location	Suffolk	Suffolk	Dorset	Kent		
Drilled	01.10.96	28.03.97	14.11.96	27.10.98		
Assessed	05.11.96	29.04.97	05.02.97	14.12.98		
Crop GS	13	13	12	13		
Insecticide seed treatment g a.i./100 kg seed						
Untreated	100	100	100	100	100	100
(Plants/m² in untreated)	(336)	(323)	(327)	(270)		
Gamma HCH 24 g	117	104	114	113	117.1	(103 - 140)
Imidacloprid 35 g	117	108	112	109	123.2	(107 - 161)

Table 2 has the results from trials where *Agriotes* damaged plants were counted. Once again, imidacloprid and gamma HCH performed similarly.

Table 2. % Reduction in number of wireworm damaged plants.

Trial	AE-22-97	BJ-21-97	EA-23-97	WR-03-97	WR-09-99	Mean	(Range)
Location	Glos.	W Mids.	Suffolk	Glos.	Herefords.		
Drilled	14.10.96	11.10.96	01.10.96	07.10.96	07.10.98		
Assessed	06.12.96	27.03.97	05.11.96	13.11.96	01.12.98		
Crop GS	14	24	13	13	14		
Insecticide seed treatment g a.i./100 kg seed							
Untreated	0	0	0	0	0	0	
(Damaged plants/m² in untreated)	(7)	(17)	(5)	(7)	(4)		
Gamma HCH 24 g	76	95	79	79	97	85.2	(76 - 97)
Imidacloprid 35 g	78	74	64	84	98	79.6	(64 - 98)

Slugs

Table 3 has the grain hollowing results derived from exposed 5 g lots of grain which were set up on the soil surface in fields where slugs were active. Two things are striking. Imidacloprid had a consistent beneficial effect even at sites where there was considerable damage to untreated grain (WR-04 and WR-15-91). There also appeared to be little rate related effect. 35 g a.i./100 kg seed was broadly as effective as the higher rates tested. No dead slugs were recovered from the trials.

Table 4 includes the emergence data from the drilled field trials, while Table 5 shows the reduction in slug feeding on the emerged plants.

Table 3. % Reduction in numbers of slug hollowed grain in field exposed 5 g lots.

Trial Location	SC-14-90 East Lothian	WR-14-90 Herefords.	WR-15-90 Herefords.	NR-02-91 Yorks.	WR-04-91 Herefords.	WR-15-91 Herefords.	Mean	(Range)
Insecticide seed treatment g a.i./100 kg seed								
Untreated	0	0	0	0	0	0	0	
(% damaged seed in untreated)	(6)	(17)	(35)	(25)	(61)	(71)		
Imidacloprid 35 g	100	7	85	75	61	77	68	(7-100)
Imidacloprid 70 g	94	86	80	76	40	68	74	(40- 94)
Imidacloprid 105 g	100	60	70	58	72	83	74	(58-100)

Table 4. Protective effect of treatments against slug attack, shown in relative crop stand.

Trial Location		NM-01-00 Lincs.	NR-01-00 Yorks.	SM-01-00 Beds.	SR-03-00 Sussex	SW-06-00 Devon	WR-01-00 Warks.	Mean %
Drilled		05.10.99	07.01.99	06.10.99	07.09.99	15.09.99	12.10.99	Relative
Assessed		15.11.99	01.11.99	01.11.99	28.09.99	30.09.99	15.11.99	
Crop GS		13	11	11	12	11	11	
Insecticide seed treatment, g a.i./ 100 kg seed	Slug bait *	% Relative	% Relative	% Relative	% Relative	% Relative	% Relative	
None	None	100	100	100	100	100	100	100
(No. of plants/m² in untreated)		(155)	(159)	(170)	(227)	(179)	(126)	
None	Admixed	116	129	118	122	124	103	119
None	Broadcast	115	126	103	113	127	84	111
Imidacloprid 35 g	None	111	108	111	119	133	86	111
Imidacloprid 35 g	Admixed	112	116	108	111	136	115	116
Imidacloprid 35 g	Broadcast	113	123	123	104	132	126	120

* Methiocarb 3% w/w applied at 5 kg/ha

Table 5. % Reduction in number of slug damaged plants.

Trial		NM-01-00	SM-01-00	SR-03-00	SW-06-00	WR-01-00	Mean
Location		Lincs.	Beds.	Sussex	Devon	Warks.	%
Drilled		05.10.99	06.10.99	07.09.99	15.09.99	12.10.99	Reduction
Assessed		15.11.99	01.11.99	28.09.99	30.09.99	15.11.99	
Crop GS		13	11	12	11	11	
Insecticide seed treatment, g a.i./ 100 kg seed	Slug bait *	% Reduction	% Reduction	% Reduction	% Reduction	% Reduction	
None	None	0	0	0	0	0	0
(No. of plants/m² in untreated)		(29)	(55)	(95)	(76)	(14)	
None	Admixed	30	68	0	26	60	37
None	Broadcast	69	83	45	43	73	63
Imidacloprid 35 g	None	13	0	1	35	0	10
Imidacloprid 35 g	Admixed	40	32	0	21	85	35
Imidacloprid 35 g	Broadcast	64	81	32	59	74	62

* Methiocarb 3% w/w applied at 5 kg/ha

DISCUSSION

Agriotes

Imidacloprid and gamma HCH both performed similarly and effectively against *Agriotes* attack on the cereals. This was confirmed whether the measurement of efficacy was based on emergence counts or based on a count of *Agriotes* damaged plants. Clearly the loss to UK agriculture of gamma HCH as an *Agriotes* seed treatment can be substituted by the use of imidacloprid.

Slugs

The original observations, from some early imidacloprid trials, that less slug damage occurred on imidacloprid treated plots, was strongly confirmed in the "exposed 5 g seed lot" tests. The data show very consistently that slugs fed less on the imidacloprid treated grain. The degree of protection was of a high order and not much influenced by rate of imidacloprid, in the range 35 - 105 g a.i./100 kg seed.

The protection revealed under these rather artificial conditions was confirmed as being of practical significance by the drilled field trials. The crop stand results (Table 4) show an almost universal benefit of imidacloprid seed treatment, which is all the more remarkable bearing in mind the difficulty of obtaining repeatable results from naturally infested slug trials.

It is interesting to compare the results of the emergence counts and damaged plant counts. The emergence data tend to reflect early, below soil surface damage. There is evidence that the seed treatment and the admixed (drilled) slug baits were more effective at this very early stage than the broadcast application (Table 4).

This might be predicted, since the a.i.s protecting the seed are at the site of slug feeding and crop damage. The efficacy of the broadcast application, with the bait on the soil surface, will depend on slugs at some time foraging above ground. Slugs which remain underground will not be affected.

On the other hand, the broadcast application shows superior reductions in slug damage to the aerial plant parts (Table 5). The location of bait on the soil surface will have more effect on those slugs active on the soil surface and feeding on the leaves.

The data suggest that the benefits of imidacloprid are stronger when the slug is in relatively close proximity to the treated seed. The effect does not persist to protect the shoot for long as it grows and concentrations of the a.i. within the plant are diluted. However, protection to the germinating seed underground is a considerable benefit. Conventional slug baits have difficulty in reaching slugs active below ground, and at this stage the crop is particularly vulnerable to slug damage. It takes very little feeding damage to destroy a plant completely by consuming the seed embryo, a particular target of slug depradation.

CONCLUSION

Imidacloprid clearly offers good protection from *Agriotes* attack. It also provides some benefit in protection from slugs particularly at the critical early stages of seedling development before the crop emerges. This is when enormous damage can be done by grain hollowing and which is very difficult for a slug bait to reduce reliably. However, it is clear that prolonged slug pressure on the crop needs to be relieved by more targeted measures such as the use of a reliably effective slug bait like methiocarb.

ACKNOWLEDGEMENTS

Thanks are due to colleagues who carried out the field work and to growers who allowed the work to be done on their land.

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Thiamethoxam - a new sugar beet seed treatment in Finland

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ABSTRACT

Thiamethoxam is a new insecticide used as a seed treatment for sugar beet, which was tested in the official plot trials in Finland in 1997-1999. Treatments with thiamethoxam 45 g and imidacloprid 60 g per seed unit (SU=100.000 seeds) resulted in the same plant number per row-meter (5.7 pcs/rm). Thiamethoxam 60 g/SU and imidacloprid 90 g/SU resulted in comparable plant stands. If no seed treatment was applied, capsid bugs damaged 29% of the sugar beet seedlings. Seed treated with imidacloprid 30, 60 and 90 g/SU showed a reduction in damage of 40, 55 and 47%, respectively. Seed treated with thiamethoxam 45 and 60 g/SU resulted in a reduction of 61 and 65%, respectively.

In summer 2000, thiamethoxam was tested in Finland on 50 sugar beet farms on approximately 250 ha. Thiamethoxam-treated seed (45 g a.i./SU) was compared with seed treated with imidacloprid 60 g/SU. The plant number per row-meter was very similar in both seed treatments. Flea beetles and capsid bugs were controlled equally by both treatments.

INTRODUCTION

Capsid bugs *(Lygus rugulipennis)* and flea beetles *(Chaetocnema concinna)* are the pests which most seriously damage sugar beet during the early summer in Finland. Flea beetles are well controlled by spraying pyrethroids, but control of capsid bugs requires an effective insecticide on the seed.

When imidacloprid was introduced on the market in Finland, it soon became the seed treatment of choice (Fig. 1). 90% of all sugar beet seed sold in Finland for summer 2000 was treated with imidacloprid 60 g/SU.

The dose of thiamethoxam recommended for sugar beet was 60 g/SU (Senn *et al.*, 1998). The aim of this study was to determine the dose of thiamethoxam needed for Finnish conditions to provide effective control against capsid bugs and to compare thiamethoxam with the commercial seed treatment with imidacloprid (60 g a.i./SU).

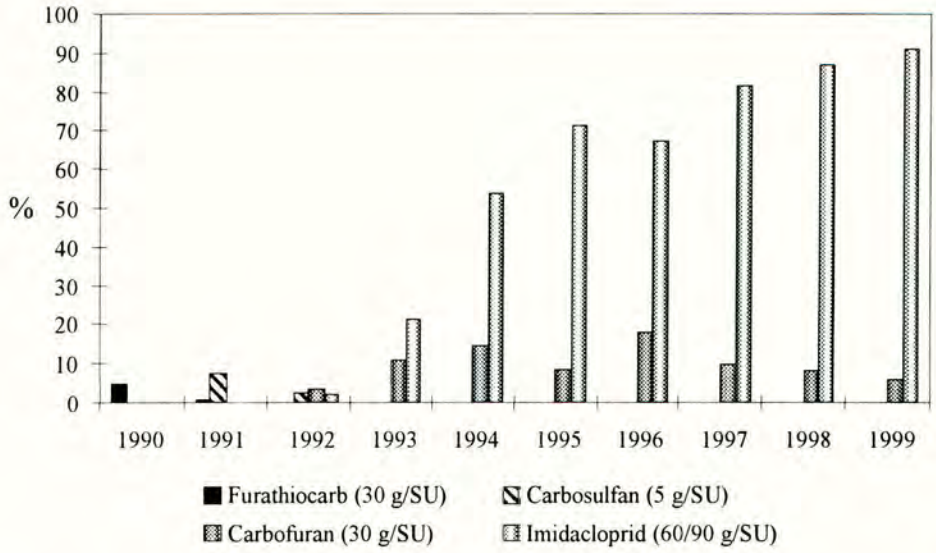

Figure 1. Percentage of seed treated with different insecticides in Finland in 1990-99.

METHODS AND MATERIALS

Plot trials were carried out between 1997-1999 on two trial farms of the Sugar Beet Research Centre. One of the farms (Laurila) is located in the middle of the Finnish sugar beet area, where capsid bugs are the main pest. The other one (Pohjankartano) is located near the coast of the Baltic Sea, where flea beetles and capsid bugs damage sugar beet most seriously in the early summer.

The doses of thiamethoxan tested were 45 and 60 g/SU. Imidacloprid 30, 60 and 90 g/SU were used as reference. The efficacy of the chemical seed treatment was assessed in relation to the untreated control.

All seeds contained thiram 4 g/SU and hymexazol 12.6 g/SU to control damping-off. The sugar beet variety was Sirkka (Novartis Seed AB) and the seed treatment was applied by Novartis Seed AB (Sweden).

The trial plots, containing 8 rows, were 10 m long and 5 m wide. Each treatment had four randomised replicates. The trials were sown and fertilised by the one-pass method in May using a seed spacing of 15 cm and a row spacing of 47.5 cm.

Annual weeds were controlled by three sprays at both locations, and couch grass was controlled using propaquizafop (100 g/ha) as necessary. The trials were hoed at the beginning of July and harvested at the end of September.

The damage caused by flea beetles was assessed from 50 beet plants per plot at the beginning of June. The percentage of beet damaged by capsid bugs at the apical meristem was assessed from 60 plants per plot before midsummer. The final plant number per row-meter was assessed from the two middle rows of the plot at the beginning of July. All results were statistically analysed using analysis of variance.

Farm Trial 2000

The farm trial was located throughout the whole sugar beet area including 50 farms and 68 fields. The seed for these on-farm trials was treated with thiamethoxam 45 g/SU ('Cruiser-45'). The varieties used were Helmi (Novartis Seed) and Centaure (van der Have). The farmers used their own commercial seed treated with imidacloprid 60 g a.i./SU ('Gaucho-60') of the same variety as reference. Both seed treatments were drilled at the same time. There were 3-4 rows of thiamethoxam treated seed and 3-4 rows of imidacloprid treated seed in the same drilling machine.

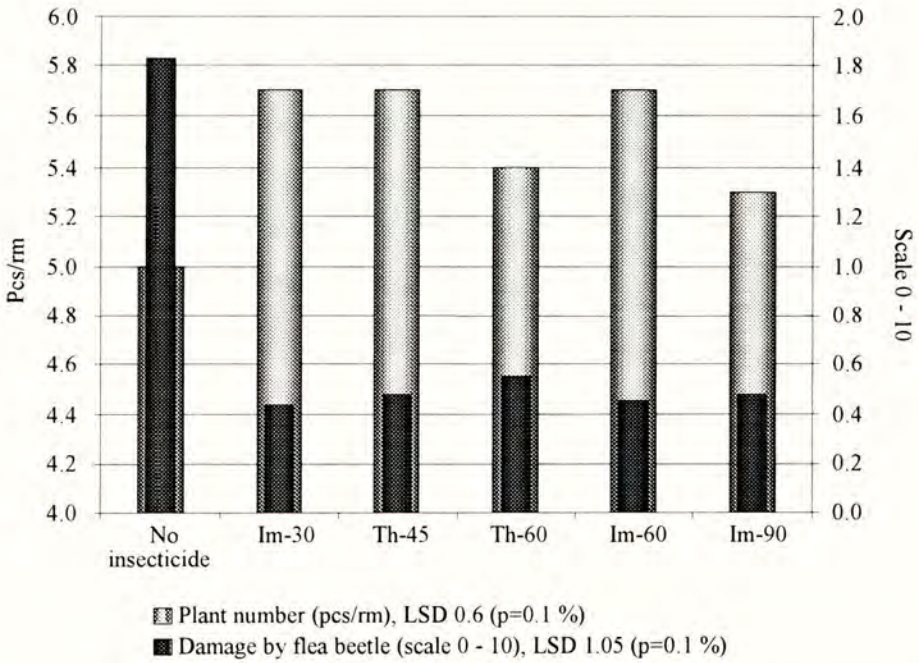

Figure 2. Plant number per row-meter (pcs/rm) and damage by flea beetles (scale 0 - 10). Scale: 0 = no damage, 10 = leaves totally eaten by flea beetles. Symbols: Im-30, Im-60 and Im-90 = Imidacloprid 30, 60 and 90 g/SU; Th-45 and Th-60 = thiamethoxam 45 and 60 g/SU, respectively.

The number of plants and the damage caused by flea beetles and/or capsid bugs were assessed from four counting areas (20 rm each). The flea beetle damage was determined

before mid-June and the other parameters at the end of June. The results were statistically analysed using Student's t-test.

RESULTS

The sugar beet was successfully established on both trial farms in 1997 and 1998. Summer 1999 was very dry at Pohjankartano, resulting in slow germination. When the final plant number was assessed at the beginning of July, the beet stand was more or less complete. The results of the plot trials are means of six trials conducted between 1997-1999.

Plant number per row-meter

If the seed contained no insecticide, the mean number of emerged plants was 5.0 pcs/rm (Fig. 2). When the seed were treated with imidacloprid 30 and 60 g/SU or with thiamethoxam 45 g/SU, there were 14% more beet per row-meter than in the treatment without insecticide. If the seed contained thiamethoxam 60 g/SU or imidacloprid 90 g/SU, the plant number was slightly lower than in the other chemical treatments, but the difference was not statistically significant.

Damage by flea beetles

Flea beetles damaged sugar beet seriously only in the summer 1997 at Pohjankartano. The mean of six trials gave a damage rate of 1.83 for the untreated control, corresponding to 3-5 spots on the cotyledon/leaf of sugar beet eaten by flea beetles (Fig. 2).

With all insecticides tested, the damage by flea beetle decreased significantly and was 70-76% lower than in beet grown without seed insecticide. The doses tested showed no clear dose response.

Damage by capsid bugs

If capsid bugs damage the meristem of sugar beet, the development of the beet is delayed. The buds at the base of the leaves or leaf primordia develop later numerous bunches of leaves (Fig. 3). These beet usually have smaller roots than the intact ones.

If the seed contained no insecticide, the damage was 29.4% (Fig. 4). Imidacloprid 30, 60 and 90 g/SU reduced the damage by 40, 55 and 47 %, respectively. When the seed treatment contained thiamethoxam 45 and 60 g/SU, the reduction was 61 and 65%, respectively. Imidacloprid 30 g/SU was significantly weaker against capsid bugs than thiamethoxam 60 g/SU.

Farm Trial 2000

Thiamethoxam (45 g a.i./SU) and the commercial seed treatment (imidacloprid 60 g/SU) gave practically the same plant number per row-meter in the farm trial. The percentages of plants damaged by capsid bugs were very similar, and there was no difference in the damage level by flea beetle between seed treatments either (Table 1).

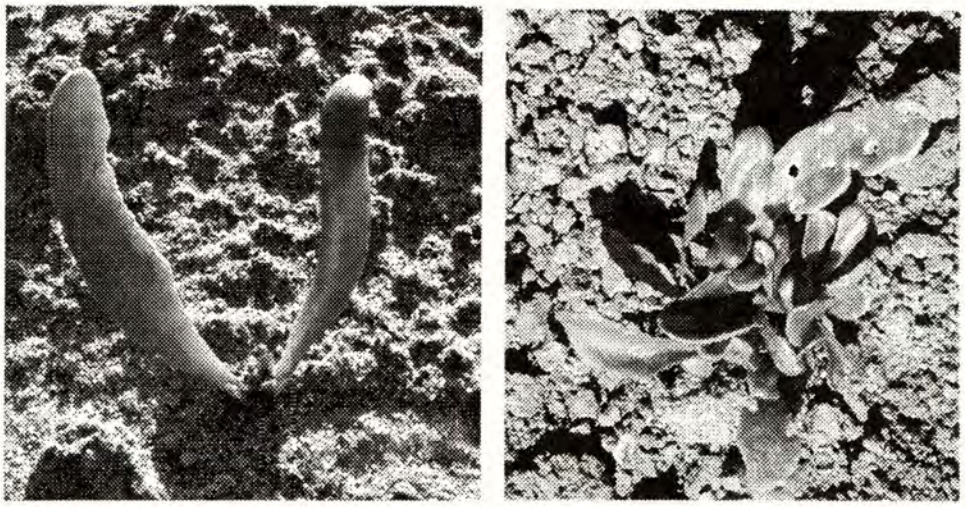

Figure 3. Early stage of meristem damage in May on the left and later stage in June on the right.

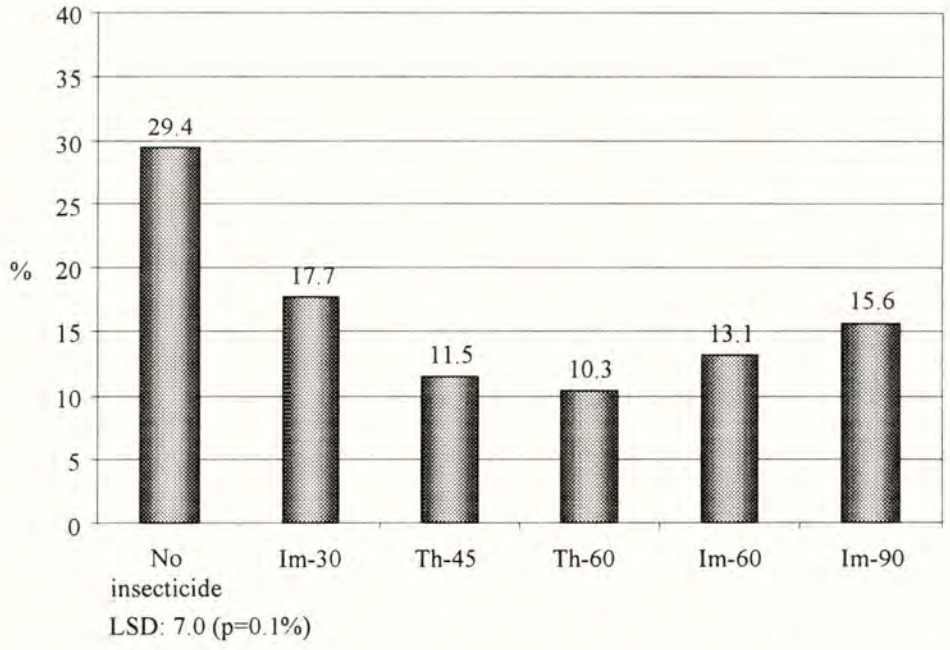

Figure 4. Percentage of beets with damaged apical meristem. Symbols: Im-30, Im-60 and Im-90 = Imidacloprid 30, 60 and 90 g/SU; Th-45 and Th-60 = thiametoxam 45 and 60 g/SU, respectively.

Table 1. Mean plant number per row-meter, mean percentage damage by capsid bugs and mean damage of flea beetles in Farm Trial 2000 in Finland. n = number of fields assessed.

Seed treatment	n	Mean Plants (pcs/rm)	Mean Damage by capsid bugs (%)	STD	n	Mean Damage by flea beetles (scale 0-10)	STD
imidacloprid 60	68	5.15 *(100.0)*	8.53 *(100.0)*	0.77	56	1.27 *(100.0)*	0.88
thiamethoxan 45	68	5.13 *(99.6)*	7.87 *(92.2)*	0.68	56	1.26 *(99.2)*	0.87

t-values: Plants (pcs/rm) 0.17
Damage by capsid bugs (%) 0.39
Damage by flea beetles (scale 0-10) 0.05

SUMMARY

In the plot trials, thiamethoxam 60 g/SU slightly reduced beet number compared to the lower dose of 45 g/SU (Fig. 2), as did imidacloprid 90 g/SU. The slower germination at the highest concentration of imidacloprid may also explain the higher damage caused by capsid bugs (Fig. 4).

In the plot trials, imidacloprid 30 and 60 g/SU and thiamethoxam 45 g/SU were equal in terms of beet number per row-meter and gave the best beet stand. This was supported by the results of Farm Trial 2000.

The control of flea beetles was very similar for all seed treatments and the small differences found in the plot trials had no significance in practice. Also the lowest doses of imidacloprid and thiamethoxam gave good protection against flea beetles. There was no significant difference between imidacloprid 30 and 60 g/SU and thiamethoxam 45 g/SU.

Capsid bugs were best controlled with thiamethoxam 60 g/SU. If the dose was reduced to 45 g/SU, the damage increased slightly (10.3 = > 11.5%). With imidacloprid 60 g/SU the damage rate was 13.1%. All treatments resulted in a good sugar beet stand.

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Chemodynamic behaviour of the new insecticide thiamethoxam as seed treatment

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ABSTRACT

The neonicotinoid thiamethoxam is a systemic insecticide. It is characterised by a low molecular weight (291.7), a low octanol-water partition coefficient (log P_{OW}:-0.13) and a relatively high water solubility (4100 mg/litre). The physicochemical parameters are favourable for an efficient uptake of the compound into the seed and transport in the xylem. During imbibition due to its high water solubility thiamethoxam is initially taken up into all seed compartments and organs of the seedling at very high concentrations. Thiamethoxam is efficiently taken up by the roots and transported in acropetal direction, it is distributed in the whole plant. Due to its high water solubility uptake of thiamethoxam is not impaired under dry soil conditions. Thiamethoxam is bioavailable over extended periods of time.

INTRODUCTION

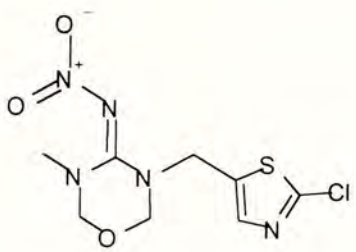
Following application of a crop protection agent as seed treatment, part of the active ingredient will be taken up by the imbibing seed. Part may be removed from the seed by water and be partitioned into the soil. Part may be lost by soil degradation and part will be taken up by the growing roots and transported into the seedling/plant. Inside the plant the active ingredient will be redistributed, translocated and metabolised. The chemodynamic behaviour of an active ingredient is determined by its chemical and physicochemical properties. In this paper we report on uptake and translocation behaviour of thiamethoxam following seed treatment.

PROPERTIES OF THIAMETHOXAM

The neonicotinoid thiamethoxam is characterised by a low molecular weight, a low octanol-water partition coefficient and a relatively high water solubility (Widmer *et al.*, 1999). The physicochemical parameters are favourable for an efficient uptake and xylem transport.

Table 1. Physicochemical properties of Thiamethoxam

Molecular weight	291.7
Melting point	139.1°C
Water solubility at 25°C	4,100 mg/litre
Partition coefficient	
n-Octanol/water at 25°C	log P_{ow} = - 0.13
Vapour pressure at 25°C	6.6×10^{-9} Pa
Hydrolytic stability	stable
Photolytic stability	labile



MATERIALS AND METHODS

^{14}C thiamethoxam (0.218 MBq/mg) was formulated either as WS 70 or FS 350 using in-house formulation techniques. Winter rape (*Brassica napus) and* summer rape *(Brassica campestris*) seeds were dressed with the FS 350 formulation, maize (*Zea mays*) and cotton (*Gossypium hirsutum*) seeds with the WS 70 formulation. The seed load was about 4µg for rape-, 1000µg for maize- and 190µg for cotton-seeds.

Remaining deposits of product on seed surfaces were removed from the seeds with 4 times 1 ml acetonitrile under ultrasonnication. For compartment analysis maize seeds were cut into halves and dissected into pericarp, embryo, endosperm, coleoptile, roots and leaf. The different compartments were ground in a hand mortar in acetonitrile. The homogenate was filtered and the residue washed with acetonitrile. Radioactivity in the filtrate was determined by Liquid Scintillation Counting (LSC). The filtrate was concentrated and analysed by Thin Layer Chromatography (TLC) using Merck 60 SI plates, 0.25 mm, F-254 with ethanol / hexane (80vol / 20vol) as solvent. Analysis of cotyledons, hypocotyls, leaves of rape and cotton was done likewise. Autoradiography was performed with dried cross-sections of seeds, dried plants and dried cross sections of pots using a BAS 1000 Fuji Bio-Imagine Analyser.

UPTAKE AND DISTRIBUTION OF THIAMETHOXAM IN MAIZE SEEDS

Seeds of *Z. mays* were treated with thiamethoxam WS 70. The seeds were germinated in 5 cm pots in commercial peat / compost / sand substrate (TKS 1). At defined times after sowing seed samples were analysed.

After sowing thiamethoxam is rapidly taken up into the maize seed, seedling and the young plant. Rather high concentrations were detected in all seed compartments. Also the coleoptile and the young leaves contained high concentrations of the active ingredient (Table 2). Maize seedlings were dissected for autoradiography as depicted in Figure 1. Thiamethoxam is readily transported in acropetal direction via xylem transport and distributed in the leaves (Figure 2), leading to systemic activity.

Table 2. Thiamethoxam concentration in different compartments of maize seedlings 14 days after sowing

Pericarp	30 ppm
Embryo	100 ppm
Endosperm	86 ppm
Coleoptile	33 ppm
Roots	2.6 ppm
Leaves	5.6 ppm

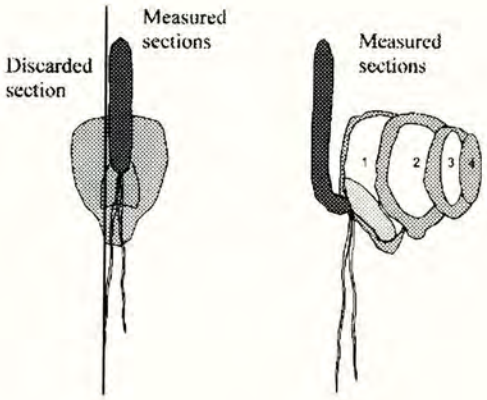


Figure 1. Maize dissection for autoradiography

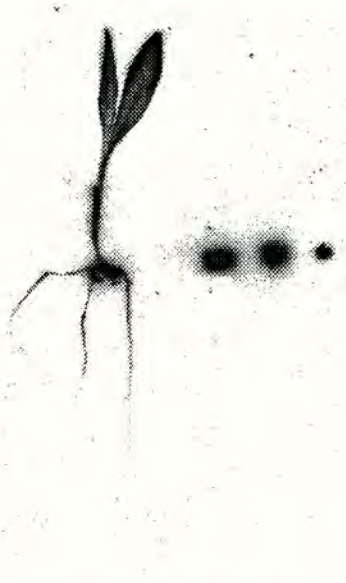


Figure 2. Autoradiography of maize sections

UPTAKE AND DISTRIBUTION OF THIAMETHOXAM IN RAPE UNDER DIFFERENT SOIL MOISTURE CONDITIONS

Seeds of *B. napus* were treated with thiamethoxam FS 350. The seeds were sown in a silt loam in a climate box at 5000 lux, 60% air humidity, 5°C during night (12h) and 10°C during day (12h). The water content of the soil was adjusted either to 30% MWC (Maximum Water Capacity) corresponding to rather dry soil conditions or to 50% MWC corresponding to normal humid soil conditions. Soil water content was readjusted every second day. At defined times after sowing seedling samples were analysed for the content of thiamethoxam in the cotyledons and leaves. Autoradiography (Figure 4) was performed with dried cross-sections of pots after cuting the plants at soil level.

Figure 3 shows that dry soil conditions did not impair uptake of thiamethoxam. In contrary, under dry soil conditions thiamethoxam was more intensively taken up into the rape cotyledons probably as a result of limited release of compound from the seeds. Highest concentrations of thiamethoxam (up to 11 ppm) were detected in the cotyledons immediately after emergence. Concentrations of thiamethoxam in the leaves were at a similar level under both soil moisture conditions indicating that on a long term the compound was equally available to uptake by the roots, due to uniform distribution of compound in the soil (Figure 4). Leaves of different age were equally supplied with thiamethoxam.

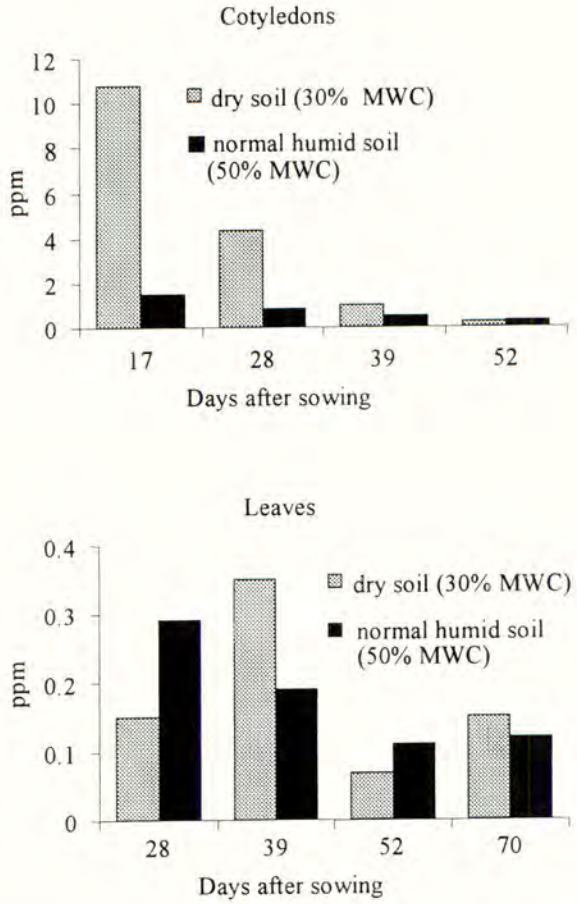


Figure 3. Concentration of thiamethoxam in cotyledons and leaves of rape cultivated under different soil water conditions

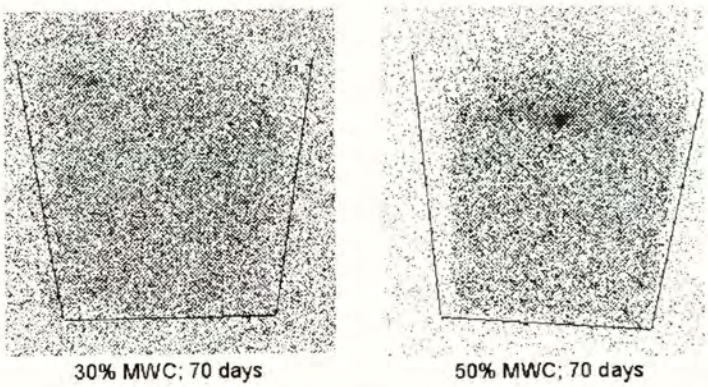


Figure 4. Distribution of thiamethoxam in the soil under different soil water conditions

UPTAKE AND DISTRIBUTION OF THIAMETHOXAM IN RAPE UNDER DIFFERENT TEMPERATURE CONDITIONS

Seeds of the summer rape (*B. campestris)* were sown in a silt loam under normal humid soil conditions (50% MWC) simulating humid spring conditions and winter rape (*B. napus*) at dry soil conditions simulating dry autumn conditions. Both varieties were cultivated both at low temperature (5°C / 10°C) and high temperature (10°C / 15°C) conditions in a climate box at 5000 lux and 60% air humidity. At growth stage 11-12 (first and second leaf developed) seedling samples were analysed for the content of thiamethoxam in the hypocotyl, the leaves and the cotyledons.

Figure 5 shows that temperature conditions did not significantly influence the concentration of thiamethoxam in the cotyledons, indicating that the different temperature regimes had no decisive effect on imbibition and initial uptake of compound. Temperature conditions however effected the concentration of thiamethoxam in the hypocotyl and in the leaves. The high temperature conditions clearly increase water transport through the vessels of the hypocotyl into the growing leaves and thus increase transport of the highly water soluble thiamethoxam. Nevertheless due to the favourable physicochemical parameters thiamethoxam is taken up and translocated in amounts sufficient for pest control also under cold conditions.

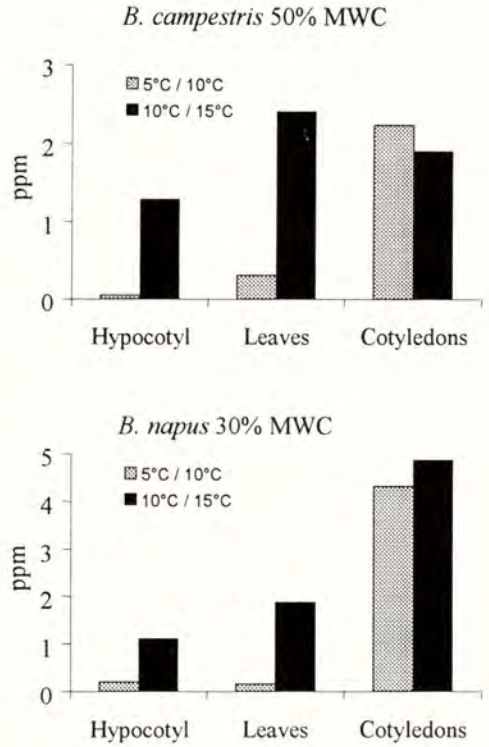


Figure 5. Temperature effect on thiamethoxam concentration in seedling compartments at growth stage 11-12

UPTAKE OF THIAMETHOXAM IN COTTON

Seeds of *G. hirsutum* were treated with thiamethoxam WS 70. The seeds were sown in a silt loam at 10000 lux, 60% air humidity, 20°C during night (12h) and 26°C during day (12h). At defined times after sowing samples were analysed for the content of thiamethoxam in the cotyledons and leaves.

Figure 6 shows that thiamethoxam was readily taken up into the cotton cotyledons leading to rather high concentrations (up to 3 ppm). In contrast to rape thiamethoxam transport into the cotyledons continued as the cotyledons expanded. Concentrations of thiamethoxam in the leaves were lower by about the factor of 10, however concentrations are high enough for efficient control of sucking pests up to 28 days (thrips) and 40 days (aphids).

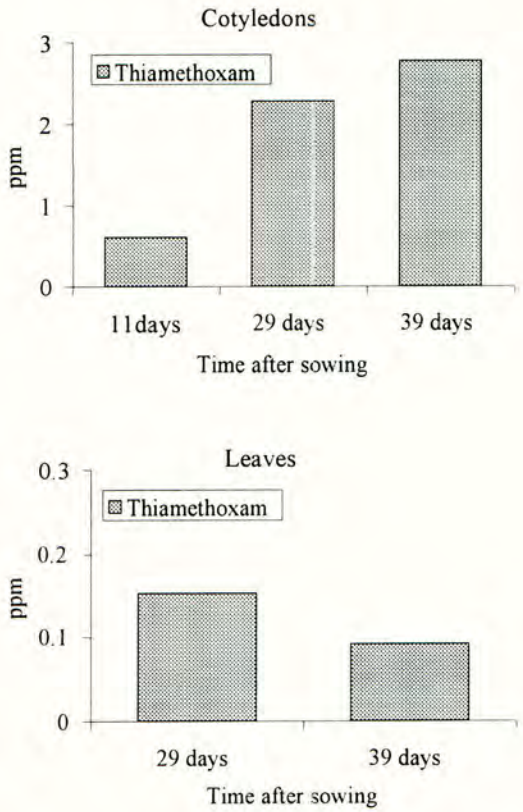


Figure 6. Concentration of thiamethoxam in cotton cotyledons and leaves

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Seed treatment – an emerging technology in agriculture in Latin America demonstrated by the development of thiamethoxam

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ABSTRACT

Thiamethoxam 70 WS was extensively tested under field conditions in Brazil on several different field crops such as wheat, barley, corn, soybean, dry bean, rice and cotton, showing in all these crops outstanding levels of insect control.

Thiamethoxam at rates varying from 17.5 to 210 g a.i./100 kg seed provided high level of control of *Metopolophium dirhodium* and *Diloboderus abderus* on wheat; *Dichelops furcatus* and *Elasmopalpus lignosellus* on corn; *Orizophagus oryzae* and *Elasmopalpus lignosellus* on rice; *Aphis gossypii* and *Frankliniella shultzei* on cotton; *Sternechus subsignatus* on soybean.

Thiamethoxam 70 WS was safe to all crops at the tested rates. Thiamethoxam 70 WS can be used in Integrated Crop Management and Integrated Resistance Management programs.

INTRODUCTION

Thiamethoxam is a new broad spectrum seed treatment insecticide developed worldwide and marketed by Syngenta AG. Thiamethoxam belongs to the new class of insecticides - the neonicotinoids. Neonicotinoids have a new mode of action interfering with the nicotinic acetylcholine receptor of the nervous system of insects. It is highly active against a broad range of chewing and sucking insects (Senn *et al.*, 1998).

Thiamethoxam has an optimum water solubility which allows it to perform well under different climatic conditions. It is well distributed in the soil and taken up by the plants under very dry up to wet conditions.

METHODS AND MATERIALS

Thiamethoxam 70 WS (Cruiser®) was extensively tested under field conditions in Brazil during the years 1993 - 1999, on several different field crops and pests such as wheat against *Metopolophium dirhodium* and *Diloboderus abderus*, corn against *Dichelops furcatus* and *Elasmopalpus lignosellus*, soybean against *Sternechus subsignatus*, rice against *Orizophagus oryzae* and *Elasmopalpus lignosellus*, and cotton against *Aphis gossypii* and *Frankliniella shultzei*.

The field trials were carried out in the most important and representative growing region for each crop in Brazil. Levels of pest incidence were always considered to be high or very high.

The field trials were designed with plot size varying from 20 to 30 m^2, 3 to 4 replicates in totally randomized blocks.

The seed treatment was done just before the sowing. Thiamethoxam 70 WS was applied in slurry. The volume of the slurry was adapted according to the crop: 500 ml of slurry/100 kg seed on cotton, corn and wheat; 300 ml of slurry/100 kg seed on soybeans; and 1.5 litres of slurry/100 kg seed on rice.

The standard formulations used were imidacloprid 600 FS, imidacloprid 70 WP, carbofuran 350 SC, carbofuran 350 TS, and thiodicarb 350 RA.

Sowing, fertilization, irrigation and other cultural practices were done according to the local Brazilian recommendations for each crop. No foliar insecticide or fungicide was used, weeds were controlled.

Insect attack was assessed by visual counting of the number of live insects per plot or number of attacked plants per plot. To measure the efficacy of the insecticides the % control was calculated (according to Abbott).

RESULTS

All results shown in this paper were generated by several official institutions in Brazil in collaboration with Syngenta S.A..

Wheat

On wheat thiamethoxam 70 WS at the rates of 17.5 to 35 g a.i./100 kg seed provided high levels of control of *M. dirhodium* and *D. abderus* (Tables 1 and 2).

Table 1. Control of *M. dirhodium* on wheat by thiamethoxam 70 WS in Campo Mourão, Paraná State, Brazil – 1998.

Treatment	Rate in g a.i./100 kg seed	% control (Abbott)		
		29 d.a.s*	48 d.a.s	57 d.a.s
Untreated**	-	14.5	60.75	10
Thiamethoxam 70 WS	17.5	100	93	90
Thiamethoxam 70 WS	24.5	100	95	93
Thiamethoxam 70 WS	31.5	100	95	100
Imidacloprid 600 FS	35	100	88	83

* days after sowing
** average number of live M. *dirhodium* per plot

Table 2. Control of *D. abderus* on wheat by thiamethoxam 70 WS in Rio Pardo, Rio Grande do Sul State, Brazil – 1999.

Treatment	Rate in g a.i./100 kg seed	% control (Abbott)		
		49 d.a.s*	63 d.a.s	91 d.a.s
Untreated**	-	12	24	8
Thiamethoxam 70 WS	24.5	67	67	50
Thiamethoxam 70 WS	35	100	83	100
Thiamethoxam 70 WS	52.5	100	83	100
Imidacloprid 600 FS	45	100	83	100

* days after sowing
** average number of live *D. abderus*/m^2

Corn

Thiamethoxam 70 WS efficiently controlled *E. lignosellus* and *D. furcatus* at rates of 105 to 210 g a.i./100 kg seed on corn (Tables 3 and 4).

Table 3. Control of *E. lignosellus* on corn by thiamethoxam 70 WS in Sertanópolis, Paraná State, Brazil – 1999.

Treatment	Rate in g a.i./100 kg seed	% control (Abbott)
		15 d.a.e.*
Untreated**	-	11
Thiamethoxam 70 WS	105	46
Thiamethoxam 70 WS	140	73
Thiamethoxam 70 WS	210	92
Carbofuran 350 SC	525	55

* days after emergence
** average number of attacked plants by *E. lignosellus* per plot

Table 4. Control of *D. furcatus* on corn by thiamethoxam 70 WS in Cambé, Paraná State, Brazil – 1997.

Treatment	Rate in g a.i./100 kg seed	% control (Abbott)
		35 d.a.s.*
Untreated**	-	90
Thiamethoxam 70 WS	105	46
Thiamethoxam 70 WS	140	69
Thiamethoxam 70 WS	210	81
Thiodicarb 350 RA	700	62

* days after sowing

** average number of attacked plants by *D. furcatus* per plot

Rice

Thiamethoxam 70 WS at the rates of 70 to 140 g a.i./100kgseed showed high activity against *O. oryzae* and *E. lignosellus* on irrigated and upland rice (Tables 5 and 6).

Table 5. Control of *O. oryzae* on irrigated rice by thiamethoxam 70 WS in Bagé, Rio Grande do Sul State, Brazil – 1997.

Treatment	Rate in g a.i./100 kg seed	% control (Abbott)
		35 d.a.s.*
Untreated**	-	6.5
Thiamethoxam 70 WS	35	58
Thiamethoxam 70 WS	70	77
Thiamethoxam 70 WS	105	85
Thiamethoxam 70 WS	140	88
Imidacloprid 600 FS	140	69

* days after sowing

** average number of live *O. oryzae* larvae per plot

Table 6. Control of *E. lignosellus* on upland rice by thiamethoxam 70 WS in Ibiporã, Paraná State, Brazil – 1999.

Treatment	Rate in g a.i./100 kg seed	% control (Abbott)		
		15 d.a.e.*	25 d.a.e.	40 d.a.e
Untreated**	-	26	39	49
Thiamethoxam 70 WS	105	82	85	86
Thiamethoxam 70 WS	140	85	85	90
Carbofuran 350 TS	140	67	66	58

* days after emergence

** average number of attacked plants by *E. lignosellus* per plot

Cotton

Thiamethoxam 70 WS as a cotton seed treatment controlled *A. gossypii* and *F. shultzei* at rates from 140 to 210 g a.i./100 kg seed (Tables 7 and 8).

Table 7. Control of *A. gossypii* on cotton by thiamethoxam 70 WS in Bandeirantes, Paraná State, Brazil – 1997.

Treatment	Rate in g a.i./100 kg seed	% control (Abbott)			
		15 d.a.s.*	20 d.a.s.	30 d.a.s.	40 d.a.s.
Untreated**	-	83	119	153	131
Thiamethoxam 70 WS	105	88	86	80	59
Thiamethoxam 70 WS	140	98	89	83	64
Thiamethoxam 70 WS	210	99	90	84	64
Imidacloprid 70 WP	140	98	85	82	62

* days after sowing
** average number of live *A. gossypii* per plot

Table 8. Control of *F. schultzei* on cotton by thiamethoxam 70 WS in Bandeirantes, Paraná State, Brazil – 1997.

Treatment	Rate in g a.i./100 kg seed	% control (Abbott)			
		5 d.a.s.*	10 d.a.s.	15 d.a.s.	20 d.a.s.
Untreated**	-	17	13	10	13
Thiamethoxam 70 WS	105	99	89	78	71
Thiamethoxam 70 WS	140	99	94	90	77
Thiamethoxam 70 WS	210	100	100	98	79
Imidacloprid 70 WP	140	99	96	93	79

* days after sowing
** average number of live *F. shultzei* per plot

Soybeans

On soybeans *S. subsignatus* was efficiently controlled by thiamethoxam 70 WS at the rate of 35 to 140 g a.i./100 kg seed (Table 9).

Table 9. Control of *S. subsignatus* on soybeans by thiamethoxam 70 WS in Passo Fundo, Rio Grande do Sul State, Brazil – 1999.

Treatment	Rate in g a.i./100 kg seed	% control (Abbott)		
		2 d.a.i.*	4 d.a.i.	7 d.a.i.
Untreated**	-	-	-	-
Thiamethoxam 70 WS	70	60	76	90
Thiamethoxam 70 WS	140	63	80	83
Thiamethoxam 70 WS	210	67	80	100
Carbofuran 350 SC	1,050	60	83	100

* days after infestation (infestation carried out 21 days after emergence)
** average number of live *S. subsignatus* per plot

DISCUSSION

Thiamethoxam 70 WS used as a seed treatment showed to be effective against a range of pests on different field crops in Brazil, while being safe to all tested crop seeds.

Thiamethoxam 70 WS provided outstanding insect control, in most situations, superior to current standards and always at lower rates of application.

Due to the long lasting activity of thiamethoxam 70 WS it is possible to replace foliar or soil insecticide applications to control early pests.

Thiamethoxam 70 WS will change the way insecticide seed treatment have been done so far in Latin America, specially on cereals and field crops. It will offer to the growers high efficacy against a broad range of pests, outstanding crop tolerance, and very favourable safety to the users and to the environment.

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Polymer film coatings decrease water uptake and water vapour movement into seeds and reduce imbibitional chilling injury

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ABSTRACT

Polymer film coatings were explored to alter water movement and alleviate imbibitional chilling injury in *Phaseolus vulgaris*. Our earlier work (Taylor *et al.*, 1992) illustrated that experimental hydrophobic seed coatings could retard water uptake and enhance germination and stand establishment in cold, wet soils. A commercial film coating formulation was developed recently by Seedbiotics, Caldwell, ID and laboratory investigations were performed at Cornell. A stress test was developed by germinating seeds in saturated rolled towels for 1 d at 5 °C, then transferring to 25 °C for an additional 7 d. A single lot of 'Nicelo' snap beans was used for all studies. Seeds were coated with the film-coating formulation, 'SB2000', at 0 to 4% weight gains. A weight gain or build-up of 0.5% and greater was found to enhance germination in the stress test. Both hydration rates and sucrose leakage decreased as coating weight gain increased. The coating acted as a physical barrier to both water uptake and water vapour movement into the seeds. When the polymer was applied onto inert polystyrene spheres, the coating showed hydrophilic and hygroscopic characteristics.

INTRODUCTION

Environmental stresses (temperature and water) have a detrimental effect on germination and seedling establishment. In particular, warm-season crop seeds are negatively influenced by low temperatures, and this physiological disorder is known as chilling injury (reviewed by Herner, 1990). There are two groups of chilling sensitive seeds: 1) those that are injured after seeds have completed germination and 2) seeds that are sensitive during the early phases of germination. This discussion will focus on the second group as the injury occurs during imbibition and is termed imbibitional chilling injury (ICI). ICI can occur in large-seeded legumes, and the degree of injury is dependent on both environmental and seed-related factors (Taylor *et al.*, 1992). Environmental factors include temperature and water availability; the greatest stress takes place at low temperatures and high water availability. Seed factors include the initial seed moisture content and seed coat permeability; seeds with low moisture content, and those that imbibe rapidly are most susceptible. This discussion will focus on seed coat permeability and its role on susceptibility to ICI.

Seed coats provide a physical barrier between the embryo and the environment. Seed coat permeability to water is an important factor in regulating imbibition rate and thus susceptibility to ICI. Genetics and plant breeding determine seed coat permeability. The semi-hard seed (SHS) characteristic in snap beans (*P. vulgaris*) was selected at Cornell's New York State Ag Experiment Station in Geneva. SHS are those that do not imbibe water within a 24 h period with an initial seed moisture < 5%, while seeds imbibe readily with >10% initial moisture (Taylor & Dickson, 1987). All moisture contents are expressed on a

fresh or wet weight basis. SHS at 8% moisture content were not injured after a 24 h exposure to cold and wet soil conditions, compared with seeds of two commercial cultivars with permeable seed coats. In recent studies, the commercial snap bean cultivar 'Hystyle' was more tolerant to ICI than the cultivar 'Nicelo' (Taylor & Kwiatkowski, 2000). The difference in ICI susceptibility was attributed to the slower imbibition rate of 'Hystyle'. Collectively, those cultivars that have permeable seed coats and imbibe water rapidly require other technologies to reduce the rapid water uptake and thus reduce ICI.

Seed coating technologies may be employed to apply polymer coatings that later regulate imbibition rates. Application uniformity is essential for a successful water barrier, as poor coverage around the hilum of beans results in a pathway for water uptake (Taylor, 1987). Film coating provides a method to achieve a continuous coating over the entire seed surface. Experimental hydrophobic polymers were coated onto bean seeds, and the hydration rate and incidence of ICI were examined. Selected coatings decreased imbibition rates, decreased ICI in a laboratory stress test and improved seedling establishment when seeds were sown in a wet soil environment (Taylor *et al.*, 1992). In addition, polymeric coatings may act as a barrier to water vapour. A coating of polyvinylidene chloride reduced moisture uptake from a high relative humidity environment, but did not inhibit germination (West *et al.*, 1985). Therefore, polymeric coatings can alter the kinetics of both water uptake and water vapour diffusion into seeds.

The objective of this study was to examine the protective effect of a commercial film-coating polymer on bean seed germination in a laboratory stress test. The efficacy of coating application rate on hydration rate and sucrose leakage was studied. In addition, the coating was tested as a barrier for water vapour diffusion from a high relative humidity environment. Finally, the hydrophilic and hygroscopic characteristics of the coating were studied on inert particles.

METHODS AND MATERIALS

A single lot of nontreated 'Nicelo' snap beans (*Phaseolus vulgaris*) seeds (Asgrow Seed Co., Twin Falls, ID) was used. The film coating formulation, 'SB 2000' (Seedbiotics, Caldwell, ID) was sprayed with an external air atomization nozzle onto seeds in a laboratory-scale-coating drum. Drying air was provided during the coating operation. After coating, seeds were equilibrated at 50% relative humidity in custom designed Plexiglas chambers with solutions of glycerol/water (Bay *et al.*, 1995). The equilibrated seed moisture content was 10% (fresh weight basis). A stress test was developed that employed placing seeds on saturated roll towels at 5 °C. The seeds were maintained in this condition for 24 h, and then transferred to 25 °C for an additional 7d. Germination was recorded at the end of the test, and only normal seedlings were used to calculate the germination percentage (Bay *et al.*, 1995). The non-stressed condition (control) was 25 °C constant for the entire test. Hydration rates were determined by submerging coated seeds in a known volume of water at 5 °C, and recording the weight increase after 4 h. The increase in seed moisture content was calculated and expressed as Δ moisture content / h. The leachate was kept from each sample, and sucrose was analyzed using immobilized enzyme technology (Lee *et al.*, 1995). Sucrose leakage was expressed as μg sucrose / g seed / h.

Water vapour uptake was determined by equilibrating seeds at 50% relative humidity (as described above). Coated seeds were transferred to a 95% relative humidity chamber at 25 °C for 4 h. The seed weight was recorded before and after the 4 h exposure and the Δ moisture content / h calculated.

The hydrophilic and hygroscopic characteristics of the polymer coating were studied in the absence of seeds as the solid particle. Polystyrene spheres (6.35 mm diameter) (U.S. Plastics Corp., Lima, OH) were used as an inert substrate. Polystyrene spheres were used due to their low affinity for water, density greater than water (specific gravity 1.05), ease of coating and relatively low cost. Water uptake was determined by submerging the coated spheres in time course studies. Water vapour uptake was determined in a similar manner as described for seeds.

RESULTS AND DISCUSSION

The laboratory stress test with 1 d exposure at 5 °C in saturated rolled towels drastically reduced germination in comparison with the 25 °C temperature (Figure 1). All coated treatments resulted in ca. 80% germination, while germination of noncoated seeds in the stress test was 32%. Germination increased in a linear manner as coating weight-gain increased from 0 to 0.5% in the stress test. Similar improvements from the polymeric coating were reported for *sh2*-corn (*Zea mays*), soybean (*Glycine max*) and cotton (*Gossipium hirsutum*) when tested under stress conditions in this conference proceedings (see Ni, 2001).

The polymeric coating altered hydration rates and sucrose leakage at 5 °C (Figure 2). A general decrease in both hydration rate (Δ moisture content / h) and sucrose leakage rate μg sucrose / g seed / h) was measured as coating weight-gain increased from 0 to 1.0%. The sucrose leakage rate was correlated with the hydration rate ($r = 0.95^{**}$). Therefore, the film-coating formulation coating retarded water uptake during the first 4 h of imbibition with a parallel decrease in sucrose leakage.

The polymeric coating's effect on water vapour diffusion was also tested by comparing the movement of water into coated seeds. Seeds equilibrated at 50% relative humidity were transferred to a chamber at 95% relative humidity. The moisture movement was greatest for the noncoated seeds (0.88 moisture content / h), and decreased sharply with lowest polymer application of 0.1% coating weight gain. There was a linear decrease ($r = -0.99^{**}$) in water vapour movement from seeds with 0.1 to 4.0% coating weight gain (0.63 to 0.43 moisture content / h). Thus the film-coating provided an effective barrier at low application rates.

The hydrophilic and hygroscopic characteristics of the film-coating polymer were tested on polystyrene spheres that acted as an inert substrate. A weight increase of ca. 5 mg was measured after dipping both the coated and noncoated spheres in water for 2-3 s (Figure 3), which was attributed to surface tension. However, the noncoated spheres showed no increase in water uptake for the remainder of the test. In contrast, the coated spheres continued to take up water over the test period. Water vapour uptake was tested in a similar manner as used for seeds. The noncoated spheres showed a small weight increase from 0.1 to 8 h, while the coated spheres took up significant water vapour over the same time period (Figure 4).

In summary, early investigations were conducted in the 1980's and early 1990's on hydrophobic coatings to retard water uptake. Though these formulations were not commercialized, these encouraging results provided the basis for the development of the film-coating formulation, 'SB-2000'. This film-forming polymer was demonstrated to reduce the incidence of ICI by decreasing hydration rate. It was assumed that the coating provided a physical barrier to water uptake attributed to the hydrophobic characteristics of the coating. Though solute leakage has been used as an indicator for the loss of cellular integrity, the leakage reduction of sucrose (the most abundant sugar in most seeds, Lee *et al.*, 1995), was not indicative of a reduction in cellular damage, since the reduction in leakage rates correlated well with the changes in hydration rates. To support this hypothesis, sucrose leakage was the same after 24-h hydration when both coated and noncoated seeds were fully imbibed (data not shown). The polymeric coating was most effective in reducing water vapour movement into the seeds. A coating of only 0.1% weight gain significantly slowed the uptake of water per hour in comparison with the noncoated seeds. The novel use of polystyrene spheres provides an inert substrate with similar weight as the seed, but without the confounding effect of seed properties on water uptake and diffusion. The coating itself has hydrophilic and hygroscopic characteristics, as well as acting as a barrier to liquid water uptake into seeds and moisture diffusion into seeds.

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Figure 1. The effect of film-coating polymer application rate on the percentage germination at 25 °C and in a stress test with a 24 h exposure at 5 °C, followed by 25 °C. Means shown with standard errors.

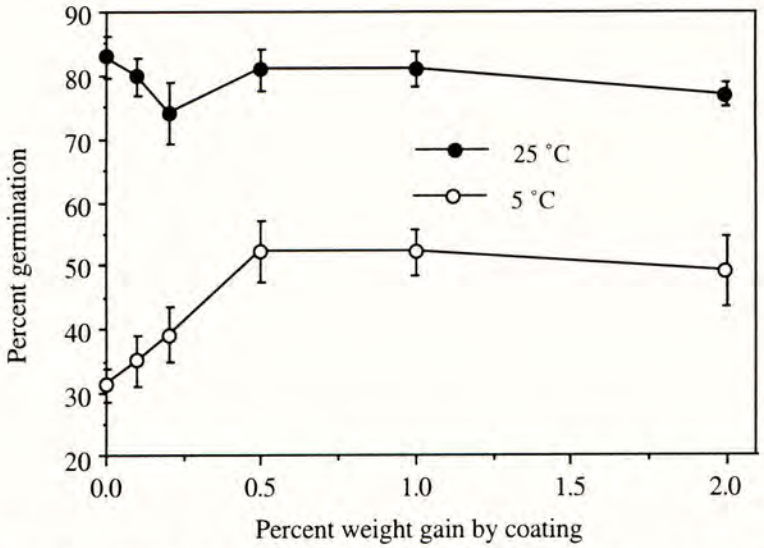


Figure 2. The effect of film-coating polymer application rate on hydration rate and sucrose leakage after 4 h at 5 °C. The hydration and leakage rates were expressed as Δ moisture content / h, and µg sucrose / g seed / h, respectively. Means shown with standard errors.

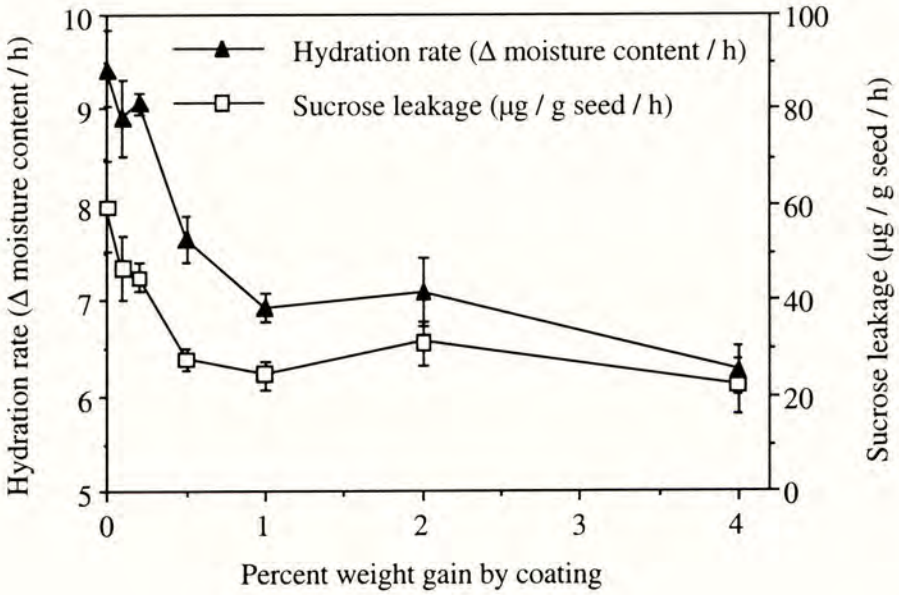


Figure 3. Noncoated or coated (4% weight gain) polystyrene spheres were soaked in water. The weight increase was recorded in a time course study. Means shown with standard errors.

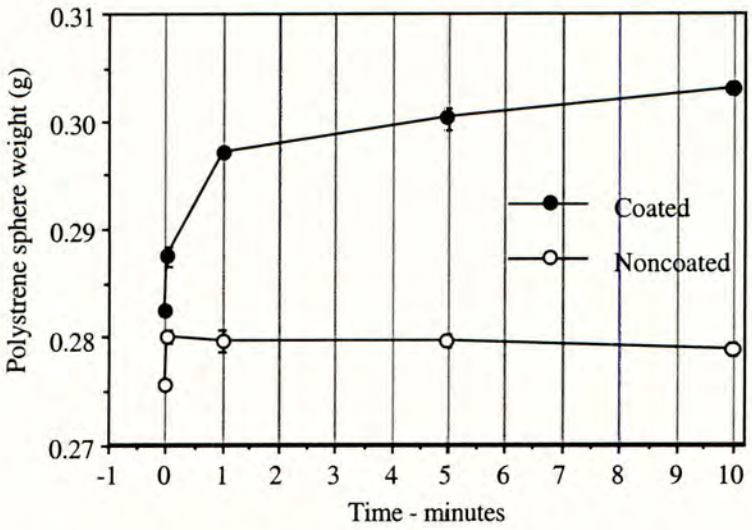
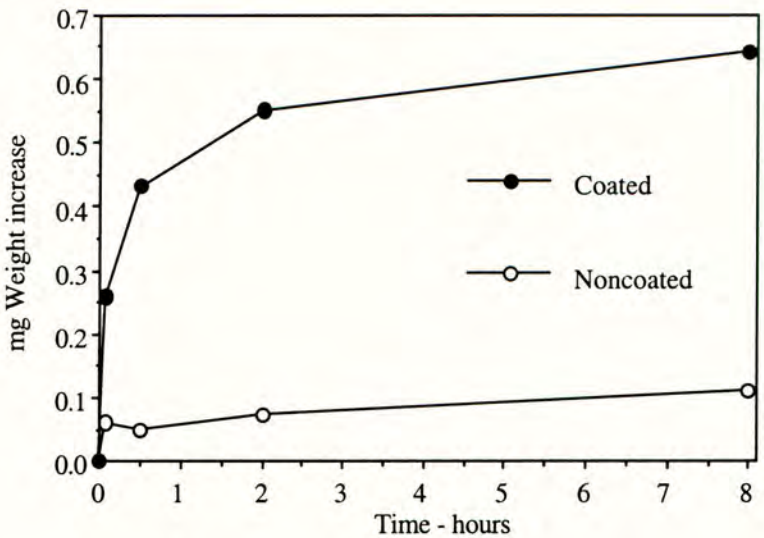


Figure 4. Noncoated or coated (4% weight gain) polystyrene spheres were transferred from a 50% to 95% relative humidity environment. The weight increase was recorded in a time course study.



Investigation of the potential of a PCR test to detect *Ustilago nuda* in barley seed

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ABSTRACT

Loose smut (*Ustilago nuda*) is a potentially serious seed-borne disease of barley. Levels of infection in seed are generally very low, since seed must meet a stringent health standard within the UK Certification Scheme. The widespread use of prophylactic seed treatments on crops for ware production against a background of low infection may become increasingly questionable. However, given the capacity of loose smut to increase, any decision not to treat seed must be supported by an appropriate seed health test result. Loose smut tests are expensive and time consuming, requiring embryo extraction and examination. Recently, DNA primer sequences for *Ustilago* species have been published, and these have been used in a preliminary investigation to determine the feasibility of a PCR test for loose smut detection in seed. The primers amplified loose smut DNA from a highly infected seed sample extracted directly from milled seed. However, this technique was not successful when applied to samples with the lower infection levels which are critical for seed treatment decisions. DNA was successfully amplified from single infected extracted embryos, and while this would offer little practical advantage over current testing methods, a bulk extraction from a large sample of embryos would considerably increase the speed of detection. The prospects for further development of this test will be discussed.

INTRODUCTION

Loose smut of barley (*Ustilago nuda*), although not a major pathogen in the UK, can cause significant loss of yield in infected crops and can spread rapidly to uninfected plants once the spores produced on smutted heads are released. The wind blown teliospores invade healthy barley florets where they produce a dormant mycelium in the embryo of the developing grain.

Control of loose smut is achieved through a combination of health standards with the Cereal Seeds Regulations (Anon., 1993), and seed treatment. All seed stocks being grown for further multiplication, and a proportion of C2 stocks are grown in check plots, and assessed for numbers of smutted ears. Any stocks failing the standard in the field may still be certified if an embryo test shows that they meet the standard or if the stock is treated by an approved product. The incidence of failures at the higher voluntary standard of 0.2% infection has been quite low (Reeves & Wray, 1994) and most seed stocks now have no detectable levels of loose smut. Embryo tests are labour intensive, and require a high degree of skill in order to identify infected material. Maintaining and assessing check plots is also labour intensive, and given the low incidence of the disease, a rapid screening method which could reliably detect an infected stock to the limits required would be advantageous.

Polymerase chain reaction (PCR) techniques are increasingly being used in seed health tests. Recently, DNA primer sequences specific for *Ustilago* species have been published (Willetts & Sherwood, 1999) which were used to detect infection in barley leaves. This paper describes preliminary investigations into the use of these sequences to detect *U. nuda* from barley seed, and discusses the potential for use of the technique in routine testing of seed for infection.

MATERIALS AND METHODS

Levels of embryo infection with loose smut were determined by soaking 1000 seeds in 5% NaOH, followed by separation of embryos in a glycerol : lactic acid : water solution, rinsing in alcohol, and a final clearing stage by boiling in lactic acid plus glycerol before microscopic examination. For the PCR tests, either 1000 seeds (high infection) or 2000 seeds (low infection) were ground to a powder in a coffee grinder. DNA was extracted using a CTAB buffer method, followed by precipitation. PCR cycling methods were carried out as described by Willits and Sherwood (1999) using the primers Uh1 and Uh4. The sequences were specific for all tested species of *Ustilago*, including *U. nuda*, but did not cross react with other common barley pathogens tested. In order to investigate whether fungal DNA could be extracted from embryos rather than whole seeds, which would significantly reduce the bulk of the material in the extraction process, DNA was isolated from individual embryos of a 68% infected seed lot at various stages of their preparation

RESULTS

Primers Uh 1 and Uh 4 amplified DNA in a sample with a high level of *U. nuda* infection, but did not amplify DNA in barley seed where no infection was recorded in the embryo test. Amplification was inconsistent in samples with a range of lower infection levels (Table 1). However, amplification was still possible after diluting the DNA from the highly infected seed sample by 1 in 2000, suggesting that levels of infection below 0.1% should be detectable (Figure 1).

Table 1. Amplification of DNA with primers Uh1 and Uh4 in barley seed with differing levels of infection with *U. nuda*.

Sample	% infection in 1000 seeds	PCR result
1	0	negative
2	0.1	negative
3	0.3	negative
4	1	positive
5	1.6	negative
6	68	positive

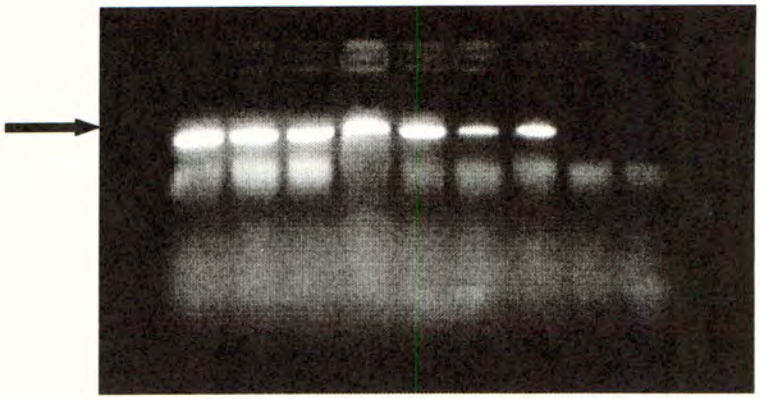

Figure1. PCR amplification of *Ustilago* specific fragment (denoted by arrow) from a highly infected seed sample at different dilutions of original sample : lane 1 = 1:10, lane 2 = 1:20, lane 3 = 1:40, lane 4 = 1:100, lane 5 = 1:200, lane 6 = 1:1000, lane 7 = 1:2000, lane 8 = 1:10,000, lane 9 = uninfected control

The PCR products of DNA extracted from 10 individual embryos at four different stages of extraction from seed are shown in Figure 2. About half of the embryos were positive in the first three stages, but none were positive after the boiling stage when DNA may have been lost into the boiling medium.

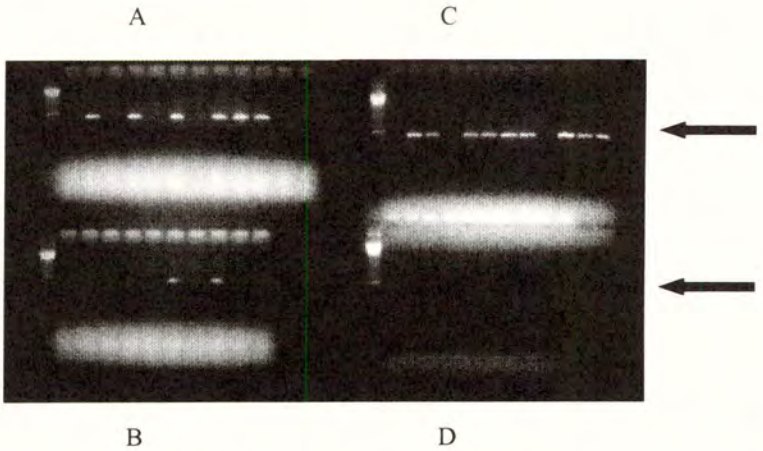

Figure 2. PCR amplification of *Ustilago* specific fragment from single embryos (1 per lane) after various stages in the extraction process. Gel set A: after 24h soak in 5% NaOH, gel set B: after rinsing in glycerol:lactic acid:water, gel set C: after rinsing in alcohol, gel set D: after boiling in lactic acid:glycerol.

DISCUSSION

U. nuda infection in severely infected barley seed can be detected by the primers Uh1 and Uh4, but it was not possible to detect the 0.2% infection limit required for certification purposes in a bulk extract, possibly due to inhibitors of the PCR in host seed material. Infection in single embryos could be detected after normal extraction, except when the material was boiled. However, PCR tests of single embryos would probably be more time consuming than microscopic examination. Work is in progress to determine whether a bulk extract of 1000 embryos would permit detection of the certification threshold. If so, the method would be more rapid than conventional tests. Quantification of the level of infection is desirable, and further work would be necessary to establish whether there is any consistent relationship between % infection and quantities of DNA detected. However, given the very low incidence of loose smut in seed stocks, a primary screen to determine presence or absence of disease would be valuable so that labour intensive microscopic examination was only carried out on positive samples.

ACKNOWLEDGEMENTS

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New technologies for seed loading and seed-to-seed distribution analysis – the critical parameters for treatment quality

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ABSTRACT

The uniform product distribution from seed to seed is a crucial application quality parameter in seed treatment. For a precise evaluation of seed to seed loading, analytical methods are the standard but require sophisticated laboratory infrastructure.

To assist the seed treatment operators and the equipment industry an optical tool for the uniform colour distribution has been used to measure the treatment uniformity at the point of application. The colorimeter Minolta CR-331 C provides the data, which is processed in a computer. Uniformity information is obtained rapidly for dressed seeds. The technology proves very promising for the assessment of the treatment quality of seed treatment dressings.

INTRODUCTION

Gradually more value is added to seeds with high performing seed treatment fungicides and insecticides. In order to develop the optimum performance for these seed treatment compounds, the treatment quality requires higher standards. These standards need to be measurable and threshold levels will be required to identify appropriate seed treatment quality.

Most common analyses are usually for bulk loadings detected with conventional GC or HPLC technologies. In centralized laboratories, the loading analysis is performed at rather high standards of analytical methods. The results are generally of high quality and reliable. However time delay from receiving a sample to the moment of producing the results does no longer permit the seed treatment operators to undertake actions to modify the treatment parameters. At the same time the information is typically just for the bulk loading. A very critical quality parameter for seed treatment is the re-distribution of the active ingredient(s) amongst the individual seeds; the single seed loading.

The quality of seed treatment requires measurable parameters. For a fast, on-site detection of the quality parameters mobile quality assessment tools are desired. Such tools may be implemented when setting up a seed treatment process in order to establish optimum application parameters. The continuing introduction of technical improvements to application equipment facilitates a range of adjustments to the treatment conditions. Choosing correct settings becomes more difficult. In the absence of vast operator experience, the possibility to evaluate the treatment quality on the site is an option to optimise the treatment quality.

The most frequently used analytical methods for loading analysis are rather precise tools but the result is fed back to the treatment site only very late in the treatment season, when large volumes of seeds are already processed. The need for an on-site analysis method is strongly desired.

Within the Syngenta Seed Treatment group some novel technologies are analysed to determine the treatment quality (bulk loading and treatment uniformity) with colour reading tools and image analysis. These methods do not require extraction and can rapidly produce loading results and/or uniformity readings.

METHOD

The key quality criteria for seed treatment are:
- Seed quality
- Sizing
- Seed surface
- Bulk loading
- Uniformity of the treatment; seed-to-seed distribution

Most of the quality parameters are inter-linked. The uniformity of a seed treatment can only be achieved when the seed lot is uniformly sized. A criterion that is very often neglected when the capacity of a treatment plant is more important than the quality. Precise seed sizing is a limiting factor in seed production. With the selection of a narrow size range of a seed lot, the seed surface area from seed to seed varies also less and therefore the treatment uniformity from seed to seed is easier to achieve. Doubling the diameter of a seed has a 4 – fold influence on the seed surface.

Analytical loading analysis for single seed loading evaluation

The single seed loading analysis is used to determine optimised application parameters. Large numbers of individual seeds are analysed to determine the best possible equipment settings. The method is time consuming and often not available. Sophisticated infrastructure is necessary particularly when active ingredients are directly analysed. Even for simplified colorimetric analysis, where a colorant is used as a tracer for the loading determination, extraction steps, filtering, diluting and reading is necessary. These steps require basic skills of a laboratory technician and at least some basic laboratory facilities.

The analytical method is still considered the standard method for the single seed loading analysis; it is the calibration tool for other methods.

Optical method for the single seed loading analysis

The optical colour measuring technique as used in the printing industry has been adapted to evaluate the uniformity of seed treatment. Called the STUNT (Seed Treatment Uniformity Test), the optical evaluation method and evaluation software was developed by SYNGENTA. The method simulates single seed loading analysis, but in an easy manner. No

extraction step is needed and results can be generated quickly. The colour of the seeds are determined and statistically evaluated. The method compares the colour difference between the average colour of an untreated seed sample with the treated sample of the same seed lot. The colour difference (Delta E) is measured. With the reading of 30 image spots, the uniformity of the treated seeds is evaluated. The colour-reading tool does nothing else but measure very precisely what the human eye also may see – the varying colour differences of treated seeds. The most common colour space L*a*b* is used (also referred to as CIELAB). In the 3 – dimensional colour space the colour difference delta E from the untreated to the treated seeds is calculated using adapted software.

The commercially available reading device is from Minolta [2]:
- Chroma-Meter CR-331C colour reading device with control unit
- Chroma Control C (D) software package for the processing of the colour data by PC

The image sampling takes place in an enclosed channel, which is completely filled with seeds. Through a glass screen readings are taken with intervals of 8 seconds. Gradually the seeds flow through the vertically placed square tube and therefore the sampling point is regularly changed. 30 readings are needed to generate the required report.
The standard deviation (sdev) of the individual readings is the tool to express the treatment uniformity.

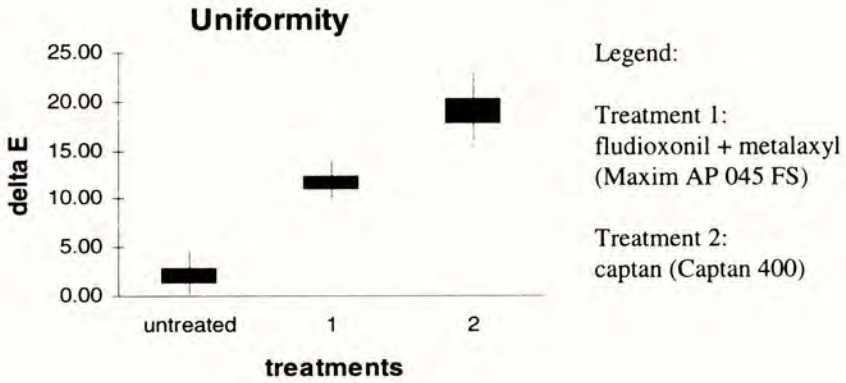

Figure 1. Reading of a sample of maize seeds treated with 2 different formulations.

The position of the square boxes indicates the colour difference to the untreated seeds; the box height indicates the uniformity of the treatment by visually demonstrating the range of colour change. A wide box reveals weaker uniformity than a narrow one.
The delta E indicates the colour difference to the untreated seeds. In this case, the Captan treatment appears in a stronger colour difference to the Maxim XL 035 FS and the Maxim AP 045 FS treatments.

The height of the box visualizes the uniformity of the treatment. It becomes obvious that the particularities of a seed treatment formulation have a strong influence on the uniformity of the treatment.

Discussion

The visual tool measures colour differences and therefore a baseline (e.g. untreated seeds) is necessary. Comparative readings are requested to calibrate the tool and to generate tolerance levels. The database is developed internally.
The visual assessment depends on the colour intensity and the thickness of the treatment film. Typical seed dressings may be easily evaluated as long as the treatment appears visually different to the colour of the untreated seeds. Heavy coatings with thick layers of products are not correctly assessed – this is a limitation to the method.
It is also useful for optimising application parameter settings. A colour-reading device is connected with a computer that converts the colour information into the desired report for uniformity.

Implementation of the colour reading technology

Particularly for the setting of application parameters in a seed treatment plant the system assists in the evaluation of the best possible treatment uniformity for seed dressings. It is a complimentary tool to the bulk loading analysis with the advantage of generating very quickly results that may be applied to optimise the treatment quality.

For the assessment of the uniformity of the treatment of seeds, the tool offers very quick and precise differences of application behaviours when treatment parameters are changed within the seed treatment equipment. The tool assists treatment operators in setting of the most appropriate equipment parameters. The readings are instantly available.

Such an electronic tool could be installed in a seed production chain where treated seeds are continuously monitored for the colour. If significant deviations occur from the target, an alarm would sound. This could be a helpful tool for an ISO 9002 certified operation.

QUALITY EVALUATION IN SEED TREATMENT - PROJECT QUEST

Image analysis – a tool for the quality analysis of treated seeds

What the human eye can identify as a variable characteristic, image analysis is the tool to quantify the information. With image analysis many physical parameters may be assessed. The uniformity of the treatment is the key objective of the assessment. Before the treatment uniformity can be assessed some basic understanding of the uniformity criteria is essential.
Uniform distribution of seed treatment products amongst a seed lot is only possible when the seed treatment equipment is capable and well calibrated, and the seed lot is clean and well sized.

Conventional assessment of the treatment uniformity is performed with the single seed loading analysis, which will remain the standard for precise analysis. The objective of the image analysis project is to obtain very quickly, and preferably on the site of the treatment, results with regard to the treatment uniformity. At the same time additional information on the seed size, shape and surface area (calculated) may be obtained.

The working principle

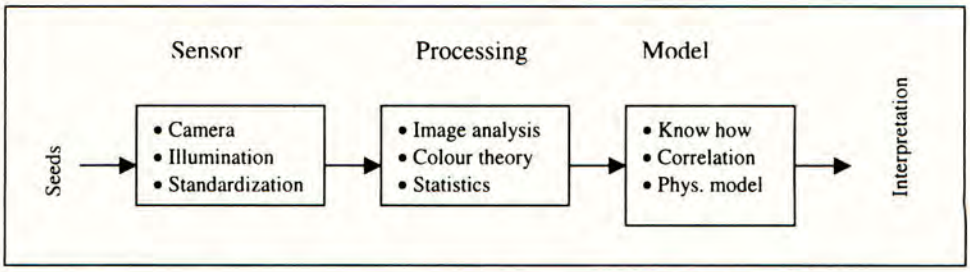

Image analysis may capture values of basic physical characteristics of the seeds.

The image analysis may capture physical parameters like the projected area. The data is processed and additional information on the shape and surface are may be obtained with arithmetic calculations.
For the assessment of the colour distribution and the uniformity of the colour appearance major challenges for the repeatability of the colour reading are necessary. Standardized illumination is only one of the criteria. The camera requires a careful calibration over the entire picture. Colour calibration is necessary.
In the initial tests the colour appearance only was determined by the hue value. The data is reproducible under controlled conditions however a more precise model including additional parameters e.g. the lightness will be evaluated.

In a simplified model the following data will be available:

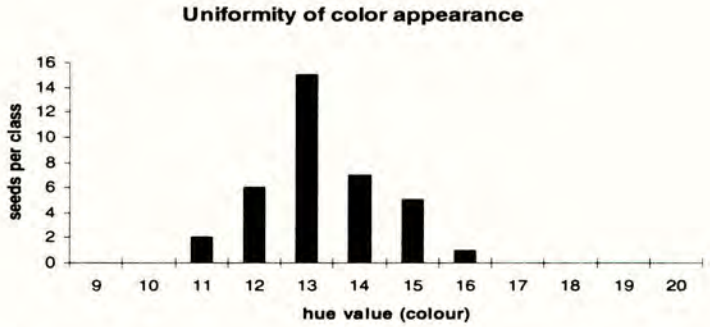

Figure 2. The hue value of seeds treated with Difenoconazole (Dividend XL RTA). The uniformity of the seeds is in a rather wide range varying from a hue value of 12 to 20. In the colour scale 1 means red and yellow has the hue value of 64, with other colour shades in-between.

The uniformity of the colour appearance is indicated in the histogram distribution where the hue values of the individual seeds are to be found.

The size information is presented as follows:

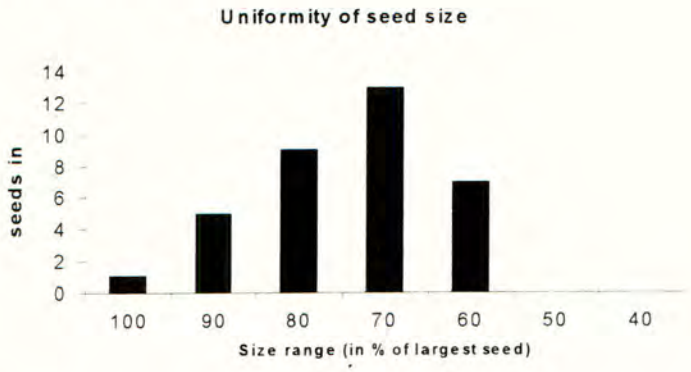

Figure 3. The size of wheat seeds is measured by the number of pixels. With varying pixels per object the size of the seed is also different. The data is presented in relative values with the largest seed considered as 100%. An absolute value will be used with a final camera setting in order to reproduce also absolute sizing information.

Summary of the image analysis

The image analysis offers quick access to quality parameters in seed treatment. The size and shape as well as the regularity of a seed treatment application may be assessed. Image analysis will not replace the standard single seed loading analysis but as a tool to optimise the application equipment and the setting parameters the technology is a great step towards more precise seed treatment application.

Equipment manufacturers and equipment operators setting seed treatment application equipment should use the system. In formulation development research the tool can help to compare the behaviour of different formulation techniques in the development stage.

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Studies on the incidence and control of Fusarium seedling blight of wheat caused by *M. nivale* var. *majus*, var. *nivale* and *Fusarium* spp. using PCR diagnostics

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ABSTRACT

The incidence of *M. nivale* and *Fusarium* spp. was determined for three seed lots produced from inoculated field trials and also in a commercially produced seed lot using traditional agar plate count techniques. Sub-species of *M. nivale* were identified by PCR assays performed on isolates of *M. nivale* recovered from agar plate tests. Seed was treated with either bitertanol plus fuberidiazole (375 + 23 g/l), fludioxonil (24.3 g/l) or left untreated. Seed was drilled at Harper Adams, Shropshire, UK and the incidence of *M. nivale* var. *nivale*, var. *majus* and *Fusarium* spp. determined on cultures produced from the stem bases of seedlings removed at the third leaf stage. The proportion of var. *nivale* and var. *majus* on untreated seedlings was similar to that in the seed although the total incidence of *M. nivale* was greater in seedlings than in seed. The incidence of the pathogens on seedlings produced from treated seed was low.

INTRODUCTION

Seed borne contamination of wheat provides the primary source of inoculum for Fusarium seedling blight. In the UK the predominant pathogen is *M. nivale* though the disease can be caused by a number of *Fusarium* spp. Early observations by Wollenweber (1931) that *M. nivale* was comprised of two sub-species were confirmed more recently by Lees *et al.* (1995) on the basis of spore morphology and by molecular characterisation using Random Amplified Polymorphic DNA (RAPD) profiles. Since the confirmation of these sub-species, Parry *et al.* (1995) reported that var. *majus* was the dominant pathogen present on wheat grain and stem bases in the UK. Simpson *et al.* (2000) showed that the accumulation of DNA by the pathogens in seedlings was greater in wheat and rye than oats. Little information, however, is available on the performance of control measures against these two important pathogens of UK cereals. The development of sub-species-specific primers towards *M. nivale* var. *majus* and var. *nivale* from RAPD markers (Nicholson *et al.*, 1996) has allowed the rapid and accurate identification of isolated pathogens. In the present study, infected seed was produced under field conditions, for use in seed treatment trials. The performance of seed treatments was assessed using the incidence of var. *nivale*, var. *majus* and *Fusarium* spp. in seed and seedlings as determined by diagnostic PCR and culture morphology respectively.

MATERIALS AND METHODS

Production and analysis of infected grain

Infected wheat grain cv. Equinox was produced according to the method described by Edwards *et al.* (1998); two field plots (2 m x 10 m each) were inoculated with spores of either *M. nivale* var. *nivale* (lot 2), *M. nivale* var. *majus* (lot 3) or *F. culmorum* (lot 4) and mist irrigated for three weeks to aid infection. Inoculated plots were separated by non-inoculated, plots without mist irrigation to reduce the spread of inoculum. The three seed lots produced (lots 2-4) together with a commercially obtained seed lot (lot 1) were used in fungicide seed treatment trials.

Two hundred seeds from each seed lot were surface sterilised in a 10% sodium hypochlorite solution for 3 min before being washed three times with sterile distilled water and plated onto potato dextrose agar (PDA) amended with streptomycin sulphate (130 mg/l) at a rate of five per plate. Plates were incubated at 20°C under near-u.v. light (12 h photoperiod) for two weeks. Colonies of *M. nivale* were removed to a fresh plate of PDA and incubated at 20°C under near-u.v. light. After one week, DNA was extracted from colonies according to a method adapted from Walsh *et al.* (1991). The supernatant produced was diluted 1:1 in a Tris EDTA buffer for use in PCR reactions. Diagnostic PCR reactions were performed using primers for *M. nivale* var. *nivale* or var. *majus* described by Nicholson *et al.* (1996). Reaction conditions were as described by the same authors for diagnostic PCR with the exception that 5 µl of template DNA was used in a final reaction volume of 25 µl. *Fusarium* spp. were identified on the basis of their colony characteristics.

Fungicide seed treatment trials

Seed was treated with either Beret Gold (a.i. fludioxonil 5 g/100 kg seed) or Sibutol (a.i. bitertanol 56 g/ 100 kg seed and fuberidazole 3.5 g/ 100 kg seed), untreated seed was used as a control. Seed was drilled at a rate of 350 seed/m^2 in plots 10 m x 2 m according to a randomised block design, four replicates per treatment were used. At the third leaf stage, thirty seedlings were removed from each plot at random. Seedlings were washed, the leaves and remaining seed coat removed, and cut into 1 cm sections before being surface sterilised as above and plated onto PDA amended with streptomycin sulphate. Three sections were placed on each plate and were incubated at 20°C for one week under near-u.v. Cultures of *M. nivale* were removed to fresh PDA plates, incubated under near-u.v. for one week before DNA was extracted and the *M. nivale* sub-species determined as described above.

RESULTS AND DISCUSSION

The incidence of *M. nivale* var. *nivale*, var. *majus* and *Fusarium* spp. (Figure 1) revealed similar frequencies of the two sub-species in seed lots 2 and 3. This occurred despite inoculation only with spores of each sub-species individually (var. *nivale* and var. *majus* respectively) and the use of non-misted, non-inoculated plots as barriers to inoculum movement. This suggests either the spread of inoculum between inoculated plots or the presence of natural inoculum.

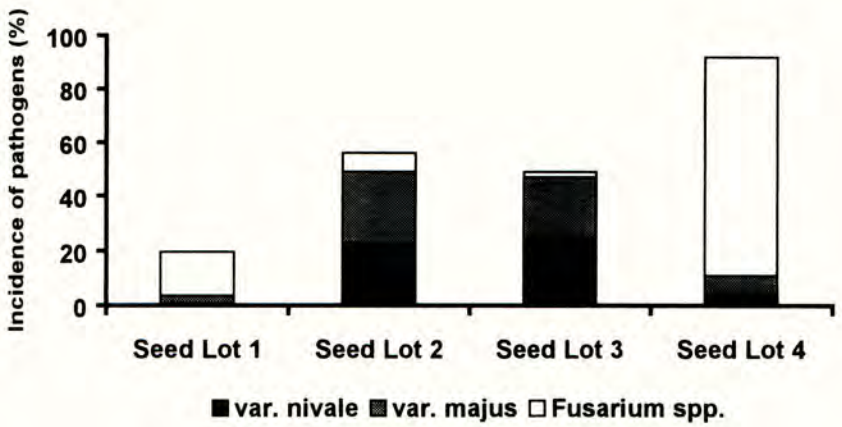

Figure 1. Incidence of *M. nivale* var. *nivale*, var. *majus* and *Fusarium* spp. in four seed lots, identified from agar plates.

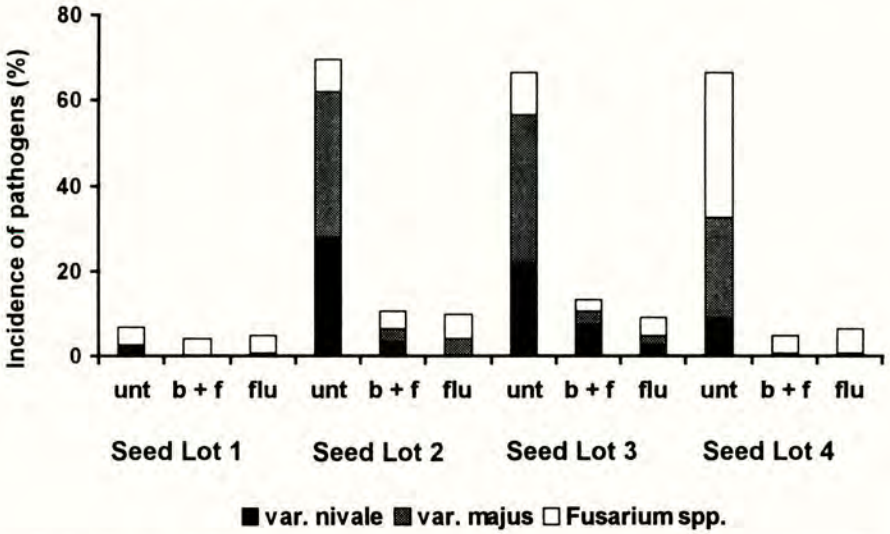

Figure 2. Incidence of *M. nivale* var. *nivale*, var. *majus* and *Fusarium* spp. in seedlings produced from treated and untreated seed. unt = untreated; b = bitertanol; f = fuberidazole; flu = fludioxinil.

Analysis of individual seeds from seed lots 2 and 3 using diagnostic PCR indicated that each sub-species was present on over 90% of 125 single seeds tested from each lot (data not shown). However, when surface sterilised seed was placed on PDA, only 49% and 47% of seed respectively were found to be infected. This suggests that surface sterilisation removes inoculum present on the seed surface or that PDA tests were not sensitive enough to detect seed borne infection in all infected seeds.

The proportion of var. *majus* and var. *nivale* isolates in the untreated seedlings was similar to that in the seed for seed lots 2 and 3 (Figure 2). This indicates that inoculum present on seed was transmitted to seedlings and suggests that little competition between isolates of var. *majus* and var. *nivale* occurred. The total number of *M. nivale* isolations from seedlings was greater for seed lots 2-4 than the incidence on seed from PDA isolations. This suggests that inoculum that is not deep seated and therefore not detected by PDA counts can lead to seedling infections. A further possibility is that soil-borne inoculum caused infections however this is unlikely as the incidence of the two sub-species on the commercial seed lot (lot 1) remained low. The incidence of *M. nivale* was greater in seedlings (33%) than in seed (11%) for seed lot 4. This further suggests the transmission of inoculum from seed to seedlings which was not detected by PDA isolations and indicates that isolates of *M. nivale* were more pathogenic under the field conditions in this experiment. The incidence of *Fusarium* spp., which were the dominant pathogens present in seed lot 4, was also greatest in untreated seedlings produced from this seed lot.

The incidence of pathogens on seedlings produced from treated seed was low indicating the effective eradication of seed-borne inoculum from those seedlings. The performance of these treatments towards the individual pathogens concerned is difficult to assess due to the low incidence of seedling infection.

ACKNOWLEDGEMENTS

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A buffer feed system to provide an even flow of potato tubers for efficient spray treatment

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ABSTRACT

A simple buffer system has been developed, in conjunction with the BCPC Potato Treater Group, to present potatoes in the optimum way for effective spraying. The buffer system is designed to provide a uniform delivery of a single layer of tubers across the full width of a roller table. An evaluation of the system was undertaken and it was shown to provide a good, even coverage of chemical without the attendant problems of over-wetting and smearing which were evident on a conventionally-configured system without the buffer in place.

INTRODUCTION

Worthwhile control of seed-borne potato tuber diseases, such as silver scurf *(Helminthosporium solani)*, skin spot *(Polysyctalum pustulans)* and black scurf *(Rhizoctonia solani)* can potentially be achieved, as part of an integrated control strategy, through the use of chemical seed treatments (Hide, 1992). However, achieving an even deposit of chemical on potato tubers, using roller table systems, is largely governed by how well the potatoes are presented to the spray and this process has been identified by Rollett *et al.* (1994) as an inefficient one.

Factors which have been identified by the British Crop Protection Council's (BCPC) Potato Treater Group as requiring attention in order to improve roller table tuber treatments include:

- Maintenance of a full roller table during treatment with a constant, regulated flow of potatoes (BCPC, 1998)
- Use of appropriate size and spacing of rollers (BCPC, 1997)
- Ensuring adequate and consistent rotation of tubers during treatment (Bishop, 1998)

It has been identified that a means of throughput regulation is normally absent or inefficient in current commercial application systems and that there is potential to use buffered feed systems to maintain a regular throughput. By keeping the table full during application, chemical losses could be reduced and wetting of rollers, which impairs rotation of tubers, minimised. A prototype buffer system was produced by E.W. Downs & Son Ltd. (Sudbury) for initial evaluation (Rodger-Brown *et al.*, 1999). This work confirmed that the addition of a

buffer to the supply can produce a more constant and even flow of tubers over the roller table. However, a number of areas for improvement and automation of the system were identified and a revised unit was built for further tests which are reported here.

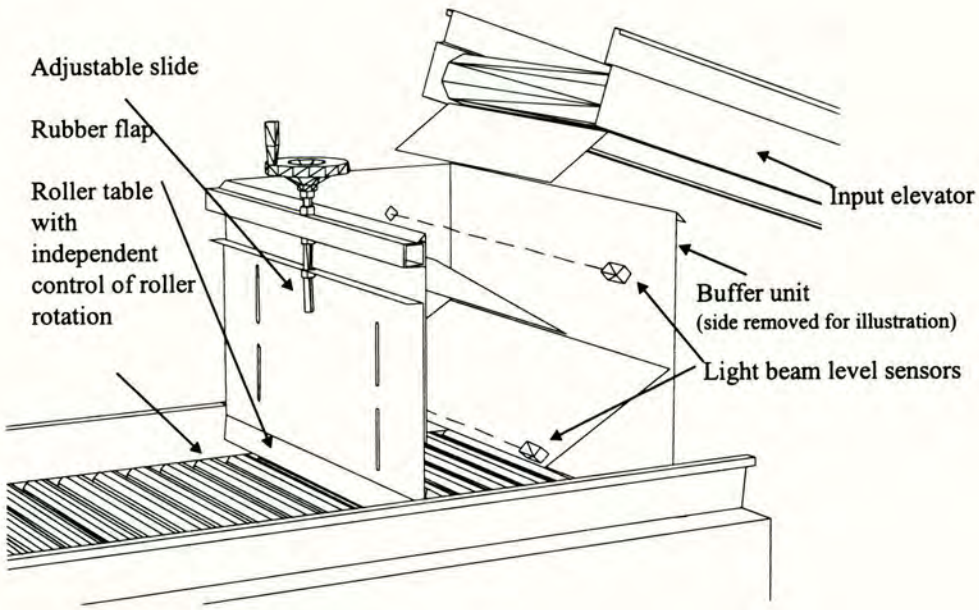
MATERIALS AND METHODS

The revised buffer system (Figure 1) included the following features:

- Flow control by high and low level sensors within the hopper
- Linkage with the sensors to stop and re-start spray output and roller table to give a co-ordinated application
- Variable timer to allow hopper to fill prior to crop release and therefore maximise the duration of each run
- Powered table rollers capable of rotating at variable speeds in either direction
- Height adjustable slide with a rubber flap attached to the lower edge, on the hopper discharge to accommodate changes in tuber size

The system was recently evaluated at the British Potato Council's Sutton Bridge Experimental Unit. It was used to apply a co-formulation of pencycuron + imazalil (250g a.i./litre + 20g a.i./litre) at 600ml/t. These chemicals are in common use as seed treatments for disease control on potato tubers. The chemical was used to treat close-graded, washed potatoes; dry-brushed crop would have been preferred but was not available.

Figure 1. Revised buffer feed system.



The trial undertaken comprised three treatments as follows. Data for each run are summarised in Table 1:

Run 1: Two nozzles, buffer operational, application matched to a full table
Run 2: Two nozzles, no buffer, application matched to a full table but table only partially full
Run 3: One nozzle, no buffer, application matched to a partially full table

Table 1. Treatments applied and throughputs measured.

Run	Number of nozzles	Buffer	Weight (t)	Time (s)	Calculated spray applied[1] (litre)	Throughput (t/h)
1	2	Yes	1.280	904	3.134	5.12
2	2	No	0.789	900	3.120	3.16
3	1	No	0.765	900	1.875	3.06

[1] calibrated output × run time

Treatments were applied using a hydraulic sprayer located in a hood (Team Sprayers, Ely). The hood had fan assistance fitted, designed to help direct the spray onto the target. Prior to application, the system was calibrated first by the loading of rollers while stationary then, having set the roller speed to achieve the target throughput, by checking the timed output with the table running. The stationary loading was achieved by filling 10 inter-roller spaces to capacity with test tubers which were then collected and weighed. Target throughput was 5 t/h, which is equivalent to 41.7 kg/30sec. 10 rollers held a tuber weight of 8.36 kg. By dividing the weight in 30 seconds by the weight per inter roller space, the number of rollers which need to pass a given point in 30 seconds is arrived at, in this case 48. Having set the roller forward speed, the table was run fully loaded, without applying treatment and the output was collected for 60 seconds. 80.5 kg was collected which equates to 4.83 t/h. The pencycuron + imazalil formulation (*Monceren IM flowable;* Bayer plc, Bury St Edmunds) was prepared by diluting 60ml of product with water to make up a total volume of 2.5 litres. Nozzle output was also calibrated; two mint green colour-coded nozzles (ref. no. 30 HAF 014-80, Lurmark Ltd, Cambridge) were used for Runs 1 and 2 operated at 2.8 bar to give an output of 104 ml / 30 seconds. This provided an application rate of 2.5 litres/t at a throughput of 5 t/h. For Run 3, a *single* mint green nozzle was operated at 3.4 bar to give 62.5 ml / 30 seconds output, providing an application volume of 2.5 litres/t at a throughput of 3 t/h. For each run the procedure used was as follows:

- Set-up was checked: Table speed was set and throughput verified. Nozzle output was matched to throughput, at the designated volume of diluted product. Spray distribution was checked using water sensitive paper.
- Each supply condition (treatment) was then run, for the same target time of 15 minutes.
- Output was sampled, at fixed points across the end of the roller table (3 x 5 tuber samples were taken after spraying for *c.* 5, 9 & 14 min). The fixed sampling points were to minimise dose variations due to location.
- Roller wetting and spray deposits were assessed visually.
- Losses to the drip tray beneath the rollers were assessed by collecting the run-off on weighed paper towels.

- Rollers were cleaned and thoroughly dried to minimise treatment to treatment carry-over effects.

RESULTS

Results are presented in Table 2. Observations noted from each run were as follows:

Table 2. Chemical loading and drip tray losses.

Run	Calculated dose applied[1] (g/t)	Sampling time (min)	Weight of 5 tubers (g)	Pencycuron deposit (g/t)	Deposit (% of dose applied)		Drip losses	
					sample	mean	(g)	(%)[2]
1	147	5	266	103	70	75	4.7	0.15
		9	206	105	71			
		14	207	122	83			
2	246	5	285	121	49	66	52.4	1.70
		9	355	155	63			
		14	237	214	87			
3	147	5	295	120	82	70	4.7	0.25
		9	301	85	58			
		14	269	105	71			
Untreated	-	-	320	<1	-	-	-	-

[1] Calculated spray applied ÷ tonnage treated *(from Table 1)* × 60 g/litre pencycuron concentration (after dilution)

[2] Percentage of chemical applied, i.e. drip loss ÷ (calculated dose × tonnage treated) assuming 1ml by volume is equivalent to 1g by weight.

Run 1: Two nozzles, buffer operational, application matched to a full table

The table rollers remained dry throughout except at the very edge due to spray depositing on the hood and running down inside, producing excess liquid on the outer tubers. The outer tubers were not taken as a part of the sample for loading analysis. The remaining tubers were evenly treated without any visual 'ringing' with the pink-coloured chemical.

Run 2: Two nozzles, no buffer, application matched to a full table but table only partially full

The rollers quickly became saturated and to such an extent that when the table was stopped chemical began to drip from them. The tubers also became saturated by the end of this run, due to the wet rollers. This test led to an uneven application of treatment on the tubers with a concentrated 'ring' of chemical around them where they touched the rollers.

Run 3: One nozzle, no buffer, application matched to a partially full table

The rollers again became saturated although this was confined to the middle of the table, due to the single nozzle not quite giving a wide enough pattern to span the full roller.

Under-treated tubers from either side of the table were not sampled for loading analysis. Concentrated rings of chemical, illustrating uneven deposition over tuber surfaces, were again apparent.

In addition, the following points were noted during the experiment:

- To achieve the desired tuber supply from the unit, a roller table with powered rollers is necessary to reduce the strain on the bottom of the buffer flap and to achieve complete roller fill. Turning off the drive to the rollers resulted in an erratic feed.
- Liquid losses were underestimated considerably in Runs 2 & 3 by only measuring the drip losses. If the free liquid on the rollers had also been measured, the differences in losses compared with the minimal loss in Run 1 would have been significant. This was very apparent from wiping down the rollers after each run.

DISCUSSION

The evaluation indicated the positive benefits that the use of a buffered feed can offer when treating potato tubers using a spray-applied chemical treatment. These included:

- Even deposition of chemical on tubers with consistently high deposit rates. This trial achieved a consistent deposit of 70% or more using the buffer. Previously, Rollett *et al* (1994) had stated that a deposit of 40-50% of dose applied was typical for roller table application.
- Dry rollers after treatment, i.e. no undesirable 'ringing' or excessive wetting of chemical on the tubers due to transfer from rollers covered with free liquid
- Low drip losses, minimising environmental impact
- A high degree of flow control, enabling close matching of crop throughput to applicator output.

In addition, the use of the buffer system (Run 1) gave better deposit recoveries than the other tests (Runs 2 & 3) despite the tubers carrying excess liquid at the edges of the table not being sampled. Run 3 deposits were perhaps a slight over-estimate as the evidently under-dosed tubers at the edges of the table were not sampled. The even distribution of chemical enhances the prospect of fungistatic compounds, for which good coverage is paramount, providing a good level of disease control on the tuber. It also reduces any possible risk of phytotoxic effects through excess deposits.

These issues are particularly important in the seed industry and it is there that this system offers the most potential benefit in terms of enhancing fungicide coverage. Where chemical is applied during grading, as is often the case in the seed sector, throughput can be inconsistent. Use of the buffer would eliminate this variability and, on the basis of this trial, should result in better treatment. Indeed, although these were limited tests, the system has demonstrated sufficient potential for it to be taken forward for commercial evaluation in the 2000/01 season.

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The relationship between season, variety and location on the incidence and severity of *Microdochium nivale* levels in winter wheat seedlots

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ABSTRACT

Analysis of wheat seed samples from six harvests (1994-1999) showed that levels of *Microdochium nivale* varied considerably between seasons. In harvests 1994-1996 both incidence and severity of infection were low where as in 1997 and 1998 infection levels were high with the majority of samples requiring treatment for *M. nivale* infection. Samples from the north and south-west were most likely to be more heavily infected with samples from the south-east showing the lowest levels of infection. 1995 was the only season not to show any regional effects. Rainfall during anthesis correlated well with infection levels accounting for 53.4% of the variation between seed-lots. However other factors were affecting infection, particularly in years where high levels of *M. nivale* are identified.

INTRODUCTION

Microdochium nivale (formerly *Fusarium nivale*) is the principle cause of pre or post emergence damping-off in UK winter wheat crops. In seasons where high levels of seed-borne infection occurs there is a significant increase in the potential for poor crop establishment particularly where the seed is sown into poor seed-beds late in the autumn. Humphreys *et al.* (1995) showed that the percentage of seed infected with *M. nivale* is linearly correlated with seedling establishment. Although the majority of wheat seed-lots receive treatment to control seed-borne diseases prior to drilling there is an increasing trend to treat seed according to need with a 5% threshold for treatment being applied. This, coupled with the increase in demand for organic seed, has meant that being able to forecast the risk of *M. nivale* in the resulting seed-lot is becoming more desirable.

Work on Fusarium ear blight, caused in part by *M. nivale* has shown that rain during anthesis is strongly correlated with development of the disease (Inglis & Cook, 1981; Snijers, 1990; Jennings & Turner, 1996). This relationship has been used as the basis of an ear blight prediction system in the United States of America aimed at control of this disease, which can be found at the following web address www.rigdetownc.uoguelph.ca/coinfo/own/fusarium/fusarium_frame.htm. This paper describes the correlation of six years seed testing results with cultivar resistance and rainfall patterns in England.

MATERIALS AND METHODS

Winter wheat samples submitted to the Official Seed Testing Station (OSTS) at Cambridge to be examined for seed-borne diseases were tested for *M. nivale* using a standard protocol. A working sample of 200 seeds was prepared and surface sterilised in a 10% NaOCL solution

for seven minutes before being placed on potato dextrose agar plates. These were then incubated at 22°C with 12 hours nUV, 12 hours dark. After incubation for five days the plates were examined for *M. nivale*, with identification being based on colony characteristics and spore morphology. Results were then expressed as percentage of seeds infected.

Prior to analysis the percentage results were converted into a disease index on a 0 – 7 scale based on infection level (with a rating of 0 = No infection, 1 = 0.5-4.5%, 2 = 5-9.5%, 3 = 10-19.5% etc.). The samples were then grouped according to county of origin and the results correlated against total rainfall (mm) for the period 9th –14th June for that region.

DISCUSSION

The level of *M. nivale* found in wheat seed lots varied greatly between years (Figure 1). In harvests 1994-6 relatively low numbers of samples were found to contain any level of infection with few samples containing levels greater than the 5% treatment threshold. In 1997 and 1998 almost every sample contained some level of *M. nivale* (96% and 99% respectively) with the majority of samples containing levels greater than the 5% treatment threshold (76% and 79.4% respectively). The 1999 harvest was unlike any of the previous five seasons in that a high number of seed-lots contained some level of infection (76%) but a much lower number had levels of infection that required treatment (15.1%).

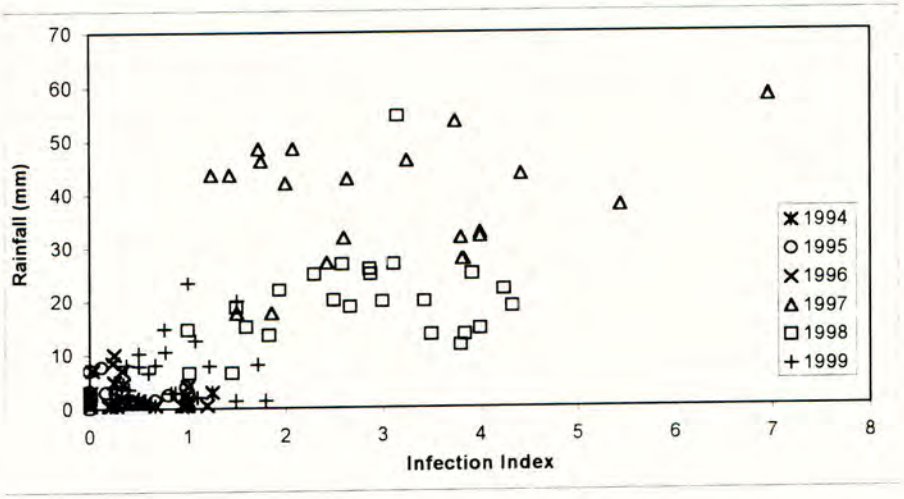

Figure 1. *Microdochium nivale* infection in wheat seed-lots from 1994 to 1999.

The level of *M. nivale* in seed-lots was found to decline with an increase in the resistance rating of the host variety (Figure 2) with this effect most clearly seen in 1997 and 1998 seasons. There is, as expected, considerable overlap between the ratings and this factor can only account for 5.8% of the variation between samples.

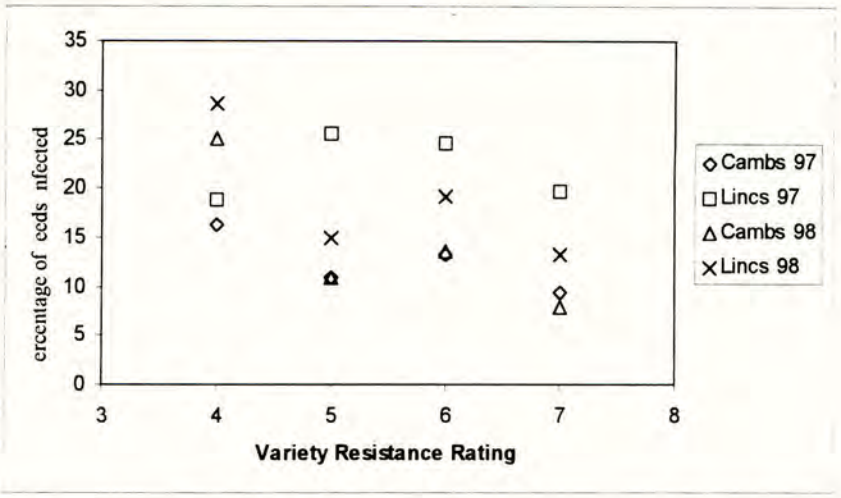

Figure 2. The effect of variety resistance rating on the mean *Microdochium nivale* infection in seed-lots from Cambridgeshire and Lincolnshire in 1997 and 1998.

The infection maps show the relative levels of infection throughout the UK (Figure 3). Even in years where relatively little infection is found in seed-lots (harvests 1994-6) there are differences between the various regions with counties in the north and south-west most likely to have produced samples containing moderate levels of infection. In 1997 and 1998 all areas produced some samples containing moderate levels of infection with the majority showing much higher levels of infection. However even in these high disease years there is significant difference between counties in the south-east such as Kent with a mean infection index of 1.46 in 1998 compared to those in the midlands such as Nottinghamshire with an index of 3.84.

Correlation between rainfall at anthesis and infection index produced a broadly linear relationship (Figure 4). Whilst rainfall during the period accounts for a significant amount of the variation (53.4%) other factors must be affecting disease levels. This is clearly illustrated when comparing the levels for 1997 and 1998 where similar levels of infection are found but much lower levels of rainfall occurred in 1998 during the period 9th – 14th June. Even when rainfall is assessed over a much longer period around anthesis there was over 30% less rain in 1998 compared to 1997. Factors such as spring rainfall or temperature may have a role in determining the level of inoculum production (Jennings & Turner, 1996) and it may be that incorporation of these factors into a model will help to explain these differences between seasons.

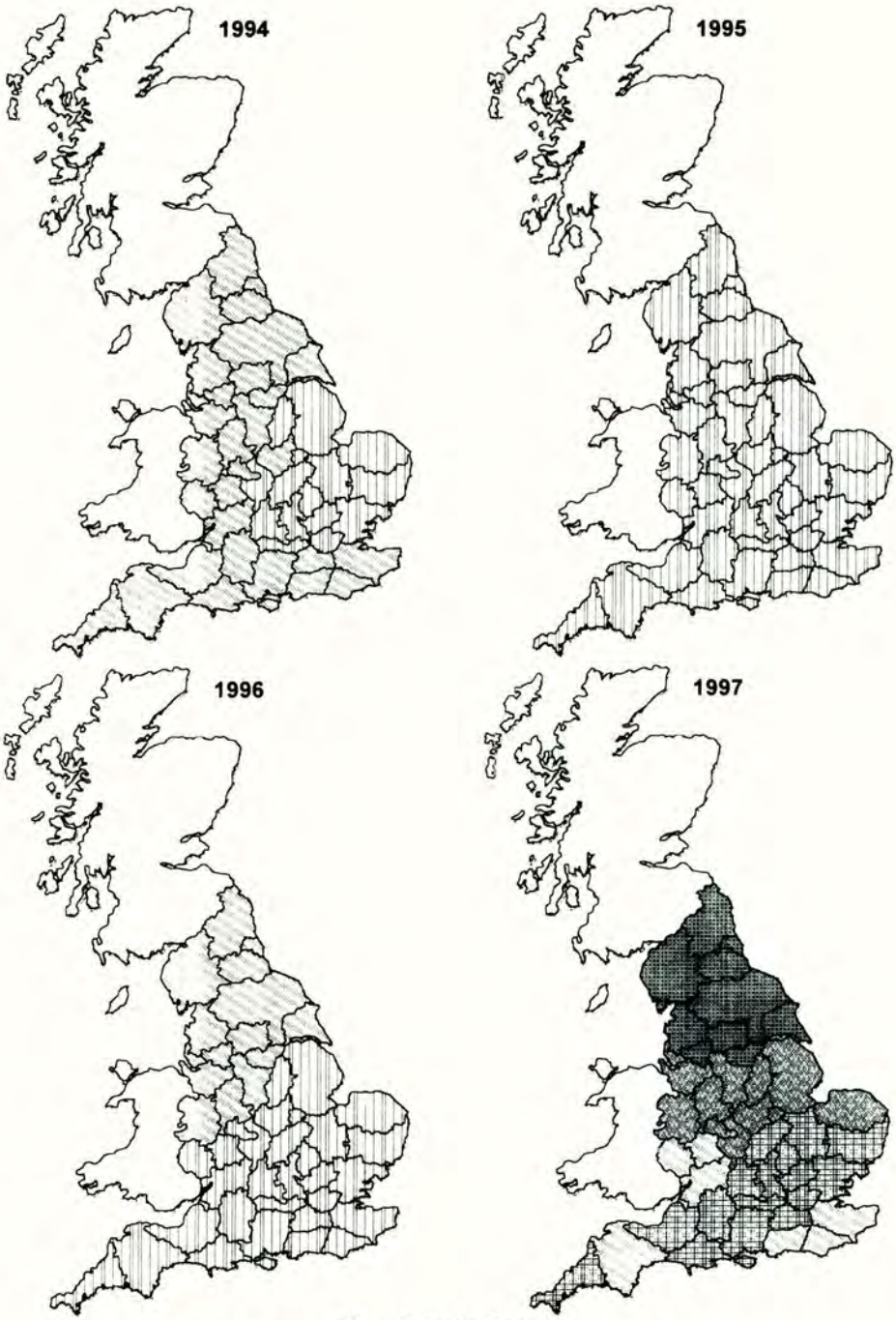

Figure 3. Regional variation in the levels of *M. nivale* in seed-lots from 1994-1999.

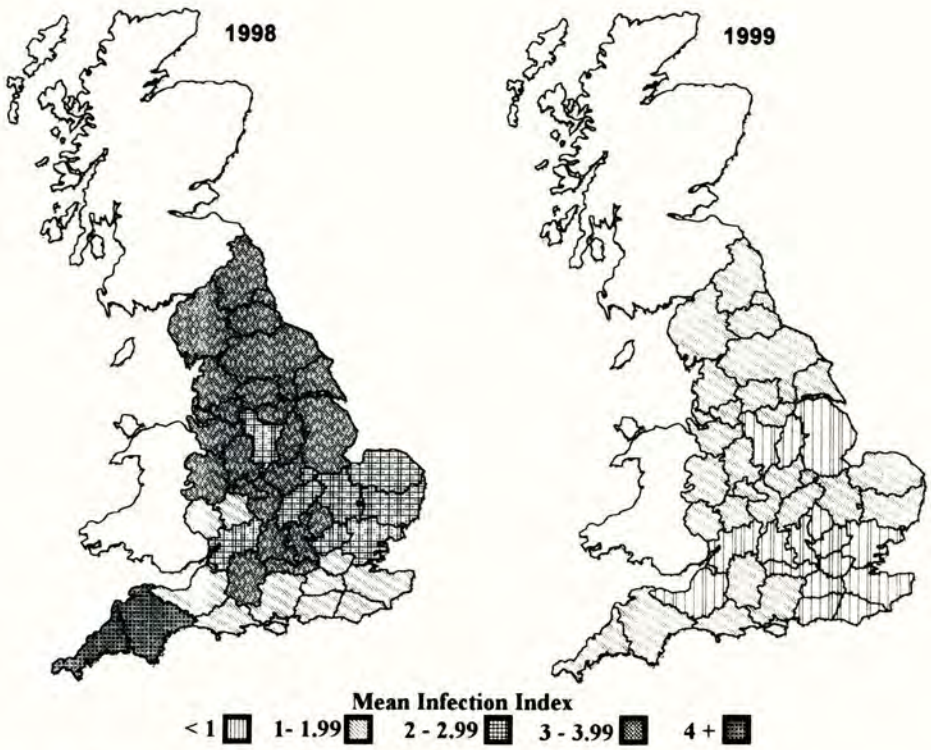

Figure 3. Continued.

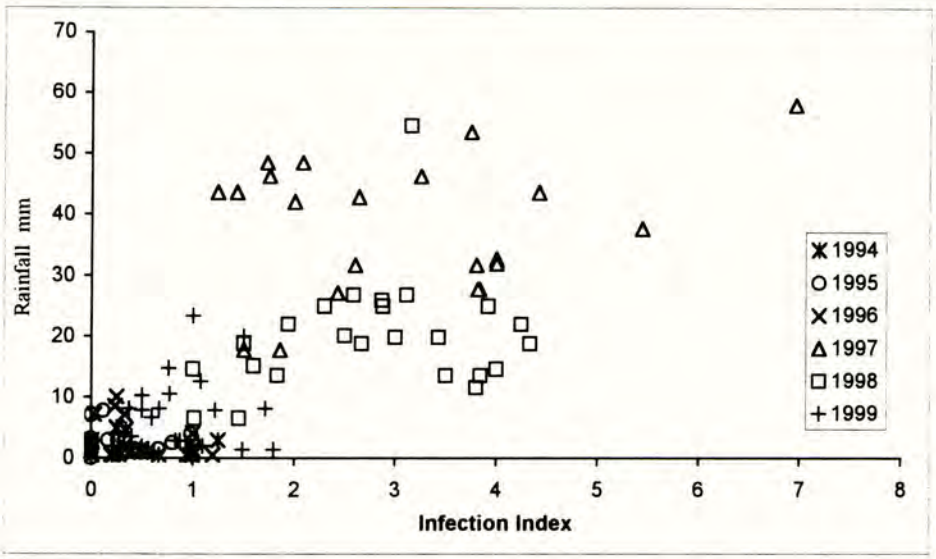

Figure 4. Correlation between mean regional infection index and rainfall during anthesis 1994 – 1999.

ACKNOWLEDGEMENTS

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Seed treatment control of seed-borne *Microdochium nivale* under different field conditions

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ABSTRACT

The performance of a range of seed treatments, for the control of seed-borne *Microdochium nivale* from a single seed lot of winter wheat, was variable across six trial sites in the UK. However, across all sites seed treatments significantly increased emergence in comparison to untreated seeds. Seed treatments did not have a consistent effect on rate of seedling emergence. Mean daily soil temperature during emergence did not explain the differences observed between sites. However, rate of emergence accounted for 62.3% of the variation in emergence at the three sites where it was recorded.

INTRODUCTION

Microdochium nivale is the major cause of *Fusarium* seedling blight of winter wheat in the UK. Controlled environment investigations have shown that *M. nivale* causes severest disease in cold dry soils (Millar & Colhoun, 1969). However in the UK, soils often have very high moisture levels during and immediately after drilling. Further controlled environment investigations have shown that rate of emergence from naturally infected seed lots was related to final number of emerged seedlings (Hare *et al.*, 1995).

The aim of this investigation was to determine if i) seed treatment performance, measured by plant emergence, was significantly different between sites, ii) rate of emergence was correlated to final emergence and iii) soil temperature at each site could explain differences in emergence.

METHODS AND MATERIALS

A single seed lot of winter wheat cv. Riband with 56% *M. nivale* infection was drilled at 6 sites throughout the UK, in November 1999, at 400 seeds/m^2. The sites were Caythorpe in Lincolnshire, Edinburgh in Lothian, Harper Adams in Shropshire, High Mowthorpe in North Yorkshire, Morley in Norfolk and Rosemaund in Herefordshire (for details of the trial sites, see Tables 1 and 2). Trials were drilled in a randomised block design with four replicates for each treatment. Treatments were Anchor® (carboxin + thiram 200 + 200 g a.i./l), Beret Gold® (fludioxonil 25 g a.i./l), Panoctine® (guazatine 300 g a.i./l) and Sibutol® (bitertanol + fuberidazole 375 + 23 g a.i./l) applied at manufacturers' recommended use rates or left untreated. Treatments were applied using a Mini-Rotostat.

At each site, plant emergence was measured at GS 10-12 and at establishment (typically GS 15-20, before tillering). Rate of seedling emergence was recorded at Caythorpe, Harper Adams and Rosemaund. Emergence was recorded every two days at Harper Adams, weekly

at Rosemaund and fortnightly at Caythorpe. Rate of emergence was calculated by: $\Sigma(n * d) / \Sigma n$ where n = number of seedlings which emerged on day d after sowing (Khah *et al.*, 1986). Days to mean emergence was the reciprocal of rate of emergence. Soil temperature during emergence at seed depth was recorded hourly using electronic dataloggers, and the mean daily soil temperature calculated.

Table 1. Information about the trial sites for each of the six locations.

Site	Date	Soil type	Sowing depth	PH	Previous crop	Cultivations
Caythorpe	10/11/99	Loam	2.5 – 3.5 cm	7.7	Spring barley	Plough, power harrow
Edinburgh	26/11/99	Loam	2.0 – 3.0 cm	5.8	Spring barley	Plough
Harper Adams	23/11/99	Sandy loam	2.5 – 4.0 cm	6.4	Potatoes	Plough, power harrow
High Mowthorpe	02/11/99	Silty clay loam	2.2 cm	8.0	Winter oilseed rape	Plough, power harrow, roll
Morley	12/11/99	Sandy loam	2.5 – 4.5 cm	8.1	Sugar beet	Plough, press, power harrow
Rosemaund	10/11/99	Silty clay loam	3.0 – 4.0 cm	7.3	Potatoes	Plough, power harrow

All data were analysed using Genstat 5. For data at the same site, ANOVAs were conducted using treatment as a factor and block as a structure. For combined data sets across all sites, there was no block structure and the data were analysed as a factorial ANOVA with treatment and site as factors.

Table 2. Rainfall and temperature data for eight weeks after drilling for the six sites.

	Accumulated rainfall (mm) in weeks 1 – 8 after drilling:							
Site	1	2	3	4	5	6	7	8
Caythorpe	*	*	*	*	*	*	*	*
Edinburgh	43.8	*	*	*	*	10.0	14.4	0.2
Harper Adams	8.6	3.2	28.0	11.2	21.8	6.2	4.8	10.6
High Mowthorpe	26.1	8.4	13.9	7.0	18.9	21.3	14.3	22.1
Morley	12.8	15.2	6.4	4.6	39.6	14.0	18.6	7.8
Rosemaund	4.0	3.8	12.4	5.4	27.4	13.6	30.2	10.2

	Average temperature (°C) in weeks 1 – 8 after drilling:							
Site	1	2	3	4	5	6	7	8
Caythorpe	*	*	7.1	4.4	3.7	0.5	2.6	2.4
Edinburgh	7.1	4.4	2.3	2.5	4.7	6.2	5.1	4.5
Harper Adams	7.0	4.9	4.3	1.0	3.0	3.4	3.1	3.6
High Mowthorpe	6.6	7.1	2.6	5.7	3.8	3.2	0.9	0.9
Morley	6.5	5.5	6.4	4.3	3.0	1.6	2.8	4.1
Rosemaund	7.2	4.2	7.0	5.3	4.2	2.0	4.0	3.9

* – no data available.

RESULTS

At all sites, seed treatments increased emergence, by approximately 30%, compared to untreated seeds (Table 3). Seed treatment performance was variable between sites. Ranking seed treatments, by percentage increase above untreated seeds, demonstrated that their

performance was not consistent across all sites. Although at five of the six sites carboxin + thiram gave the greatest final emergence at GS 15-20. Seed treatments were most effective at Rosemaund and least effective at High Mowthorpe (measured by percentage increase in emergence over untreated seeds). Treatment, site and the interaction between treatment and site all had significant effects ($P < 0.001$) on emergence. Linear regression analysis showed a good positive correlation ($R^2 = 81.4\%$; $P < 0.001$) between emergence (GS 10-12) and establishment (GS 15-20).

Table 3. Effect of seed treatments and site on emergence (m^2) at GS 10-12 of a winter wheat seed lot with 56% *Microdochium nivale* infection.

	Number of emerged seedlings (m^2)						
	Site						
Treatment	1	2	3	4	5	6	COMB
untreated	213	195	211	190	159	159	188
carboxin + thiram	289	284	304	241	253	260	272
fludioxonil	262	243	269	229	252	241	249
guazatine	267	253	265	237	229	263	252
bitertanol + fuberidazole	263	248	276	247	213	251	249
LSD (P = 0.05)	30	49	30	18	38	41	35
%cv	7.6	13.1	7.4	5.2	26.7	11.4	10.2
Degrees of freedom	19	19	19	19	19	19	119

Site 1 – Caythorpe, 2 – Edinburgh, 3 – Harper Adams, 4 – High Mowthorpe, 5 – Morley, 6 – Rosemaund, COMB – combined data for all sites.

Rate of emergence was only measured at the Caythorpe, Harper Adams and Rosemaund sites (Table 4). Site and treatment had a significant effect ($P < 0.001$) on rate of emergence however, there was no evidence of an interaction between the two ($P = 0.101$). There was a significant correlation ($R^2 = 68.8\%$; $P < 0.001$) between rate of emergence and emergence at GS 10-12 following linear regression analysis. Across all sites there were no significant differences ($P < 0.001$) between treatments. At Caythorpe, seedlings from guazatine and bitertanol + fuberidazole treated seeds emerged significantly quicker than seedlings from untreated seeds. At Rosemaund, seedlings from guazatine treated seeds emerged significantly quicker than seedlings from untreated seeds.

Table 4. Effect of seed treatments and site on days to mean emergence for a winter wheat seed lot with 56% *Microdochium nivale* infection at Caythorpe, Harper Adams and Rosemaund.

	Days to mean emergence			
	Site			
Treatment	Caythorpe	Harper Adams	Rosemaund	COMB
untreated	38.6	33.4	34.8	35.6
carboxin + thiram	37.5	34.0	32.6	34.7
fludioxonil	37.9	32.2	32.0	34.0
guazatine	35.5	32.4	31.7	33.2
bitertanol + fuberidazole	35.7	32.5	32.5	33.6
LSD (P = 0.05)	2.5	1.9	2.0	1.9
%cv	4.4	3.7	4.0	3.9
Degrees of freedom	19	19	19	59

There was no consistent relationship for temperature (seven day average temperatures after drilling or number of days below zero during seedling emergence at each site) and final emergence. However, number of emerged seedlings across all sites from carboxin + thiram (R^2 = 63.0%) was significantly correlated with average temperature in days 21 - 26 after drilling and number of emerged seedlings across all sites from guazatine (R^2 = 79.7%) was significantly correlated with average temperature in days 0 – 6 after drilling.

DISCUSSION

Site had a significant effect on plant emergence from *M. nivale* infected seed and on seed treatment performance. However, soil temperature did not clearly explain the differences observed between sites. It is probable that variables that were not measured such as soil moisture, had specific effects on *M. nivale*, wheat and the seed treatment at each site. In pot trials, increases in soil pH from 4.8 to 7.5 reduced *M. nivale* disease index and the percentage reduction in wheat dry weight (Millar & Colhoun, 1969). The same authors also reported that increases in sowing depth increased *M. nivale* disease severity. Increases in sowing depth also delay the time for emergence (Kirby, 1993). Therefore, measuring temperature alone may not adequately explain the differences observed. This indicates that seed treatments may have had an effect on overwintering survival of seedlings.

Rate of emergence was significantly correlated to final emergence demonstrating that this relationship occurs under field conditions for naturally infected seeds as well as in controlled environments. Seed treatments had a variable effect on the rate of seedling emergence, this is again probably due to the influence of environmental variables not measured.

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The interaction between ear sprays and seed treatment for the control of Fusarium seedling blight in wheat

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ABSTRACT

The results from two field trials, using four different fungicide ear sprays on inoculated mist-irrigated plots of wheat, showed that Fusarium ear blight could be controlled but the degree of control was dependant on the pathogen infecting the ears and the fungicide used. Fungicidal control of the pathogen on the ear in turn affected the amount of seed infection. This in turn had an effect on subsequent seedling emergence when seeds were drilled, with or without a seed treatment, in the field. The amount of *M. nivale* seed infection significantly influenced the emergence of seedlings in the second year but not in the first. It was found that the higher the seed infection with *M. nivale*, the lower the emergence. These results show that Fusarium seedling blight of wheat can be controlled by the use of both effective fungicide ear sprays in an infected seed crop and fungicide seed treatment of infected seed.

INTRODUCTION

Fusarium ear blight (FEB) has been widespread throughout the UK cereal crop and is recognised to be a significant but sporadic threat to wheat production (Parry *et al.*, 1995). *Fusarium culmorum* has been demonstrated to significantly reduce individual grain weight (Hare *et al.*, 1999). However, in contrast to *Fusarium* spp., *M. nivale* infected grains are often indistinguishable from non-infected grains with respect to size and colour (Hare, 1997). In addition, infected grain can be an important source of inoculum for Fusarium seedling blight if infected seed is sown in conditions favourable for the disease (Colhoun, 1970; Hare *et al.*, 1995). Grain infection has increased in frequency in the UK over the last 30 years and in a 1992/93 survey of wheat seed, all samples tested were infected with *M. nivale* (Reeves & Wray, 1994). It is clear, therefore, that in commercial crops of wheat, complete control of FEB is not being achieved.

MATERIALS AND METHODS

Field based experiments were designed and executed in the harvest years 1998 and 1999 with the aims of investigating the chemical control of FEB and seedling blight. The first trial was performed in plots of spring wheat cv. Chablis. The plots received ear sprays of Amistar (azoxystrobin 125g a.i./ha or 250g a.i./ha), Folicur (tebuconazole 125g a.i./ha) or a mixture of Derosal WDG (carbendazim 250g

a.i./ha) and azoxystrobin (125g a.i./ha) either pre or post-inoculation. Each treatment was replicated six times within a randomised block design.

In the second year a similar experiment was performed using winter wheat cv. Equinox, the ear sprays were, azoxystrobin (250g a.i./ha), Caramba (metconazole 90g a.i./ha) and a mixture of azoxystrobin (125g a.i./ha) and metconazole (45g a.i./ha) all applied pre-inoculation and replicated four times.

In both years the plots were inoculated at GS 65 (mid anthesis). In 1998 the pathogens were applied as a water-based suspension of *F. culmorum* and *M. nivale* in equal proportions. In 1999, the pathogens applied were either *F. culmorum* only, *M. nivale* only or an equal mixture of both pathogens. The spore suspension was applied with a hand held plot sprayer, at the rate of 100,000 spores per ml in 33ml of water per m^2. The *F. culmorum* spore suspension contained five pathogenic isolates of *F. culmorum* in equal proportions. The *M. nivale* suspension contained five pathogenic isolates of *M. nivale* var. *majus* and five isolates of M. *nivale* var. *nivale* in equal proportions. The mixture of the two pathogens contained 50% of each of the mixtures above. After inoculation, plots were mist irrigated for 21 days.

Seed infection, quality assessments and seedling emergence

Seed infection was assessed by counting the number of seeds from which isolates of *F. culmorum* and or *M. nivale* were recovered. For *F. culmorum*, moist blotters were used and for *M. nivale* benomyl amended potato dextrose agar (PDA) plate tests were used in accordance with the International rules for seed testing as laid down by the ISTA (1985). A bulk sample of seed for each field trial treatment was produced by taking a sample of seed harvested from field plots and amalgamating the samples from individual plots. From these bulk samples, two lots of 400 seeds were taken at random and used for the moist blotter and the PDA tests respectively. Four replicates of 100 seeds were used in each treatment.

Seed viability was assessed using the tetrazolium viability test in accordance with the International rules for seed testing as laid down by the ISTA (1985). Two hundred seeds were sampled from each field trial treatment's bulk sample and tested for viability.

A sample of seed from each of the treatments was drilled at 400 seeds m^2 in the autumn of the harvest year. Seeds were drilled into plots according to a randomised block design with four replicates. Each treatment was drilled with or without Beret Gold (fludioxonil 5g a.i./100kg seed) seed treatment. Subsequent seedling emergence was assessed by counting the number of emerged seedlings per metre of row at points within each plot. Data for seedling emergence were expressed as a percentage of seeds sown.

Statistical analysis

ANOVA was carried out on all data using the statistical software package Genstat 5.1 (Lawes Agricultural Trust. IACR. Rothamsted. UK)

RESULTS

1998 field trials

Results for the first ear blight trial conducted in 1998 showed that diseased ears were found in all plots (data not shown). Seed viability ranged from 74.4% - 88.5% with no significant ($P < 0.05$) differences between treatments. *Fusarium culmorum* and *M. nivale* were found to have infected grain in each of the samples tested from the trial (Table 1). Significant differences were observed between the untreated and some azoxystrobin treatments. Overall, the subsequent seedling emergence in the field was very poor. No treatments gave good emergence but the addition of a seed treatment did significantly ($P < 0.05$) increase emergence (Table 1).

Table 1. Seed quality results for Fusarium ear blight trial in spring wheat cv. Chablis inoculated with *F. culmorum* or *M. nivale* at anthesis and seedling emergence from seed treated with or without fludioxonil.

			Seed quality			Emergence	
Foliar treatment	rate g a.i./ha	appln. timing	Seed Viability %	*M. n* %	*F. c* %	Seed with No treatment %	Seed Treated with Fludioxonil %
Untreated			86.0	12.8	24.0	21.5	23.3
Az.	125	Pre	74.0	4.2	30.0	22.8	22.5
Az.+ Carb.	125+250	Pre	76.5	4.8	35.0	22.6	26.1
Az.	250	Pre	74.5	5.0	34.0	21.0	23.1
Teb.	125	Pre	76.0	10.2	19.0	23.6	24.0
Az.	125	Post	80.5	10.2	36.0	24.7	25.8
Az.+ Carb.	125+250	Post	79.5	10.5	29.0	23.7	20.6
Az.	250	Post	88.5	7.2	30.0	24.4	25.6
Teb.	125	Post	76.5	12.5	22.0	22.5	24.3
		LSD	N/A	4.8	5.2	0.9	
		SEM	3.6	0.8	1.8	0.3	
		CV	6.5	20.0	11.0	2.6	

Az. = azoxystrobin Teb. = tebuconazole Carb. = carbendazim
Pre = pre-inoculation Post = post-inoculation
F. c = *F. culmorum* *M. n* =*M. nivale*

1999 Field trials

Results for the second ear blight trial conducted in 1999 are given in Table 2. Azoxystrobin significantly ($P < 0.05$) reduced seed infection by *M. nivale* in all

treatments when compared with the untreated. However, it did not reduce *F. culmorum* infection. Conversely, metconazole significantly reduced seed infection by *F. culmorum* but not *M. nivale*. Overall the azoxystrobin + metconazole mixture resulted in the lowest seed infection. Analysis of seed viability showed significant ($P < 0.05$) differences in viability with respect to the pathogen used to inoculate ears and the fungicides applied to the ear (Table 2).

Overall, treatment with fludioxonil resulted in a significant increase in seedling emergence when compared with no seed treatment (Table 2). The greatest number of seedlings emerged from seed with low seed infection. Significant ($P < 0.05$) differences were seen in emergence between seed lots from plots treated with different ear sprays. More seedlings emerged when ears had received a fungicide spray to control ear disease and subsequent seed infection. Where no fungicide ear spray was applied, seedling emergence was lower even following seed treatment.

Table 2. Seed quality results for Fusarium ear blight trial in winter wheat cv. Equinox inoculated with *F. culmorum* and/or *M. nivale* at anthesis and seedling emergence from seed treated with or without fludioxonil. Foliar fungicides applied pre-inoculation.

			Seed quality			Emergence	
Foliar Treatment	Rate g a.i./ha	Inoculum	Seed Viability %	*M.* %	*F.* %	Seed with no Treatment %	Seed with Fludioxonil %
No fungicide		*F.* + *M.*	64	14.1	24.8	43.2	63.0
Az.	250	*F.* + *M.*	68	6.6	26.4	54.6	67.1
Az.+Met	125+90	*F.* + *M.*	75	4.3	20.7	54.3	69.0
Met.	180	*F.* + *M.*	60	19.7	16.6	38.4	65.4
No fungicide		*M.*	66	28.9	17.2	29.1	66.0
Az.	250	*M.*	75	16.1	12.9	62.7	70.2
Az.+Met	125+90	*M.*	84	14.6	12.4	62.4	66.0
Met.	180	*M.*	78	17.2	9.3	42.6	72.8
No fungicide		*F.*	81	19.2	24.1	40.2	60.7
Az.	250	*F.*	54	10.2	26.7	45.3	60.0
Az.+Met.	125+90	*F.*	60	11.9	18.0	49.5	70.8
Met.	180	*F.*	76	18.4	16.1	42.6	70.5
		LSD	4.3	5.7	6.0	15.7	
		SEM	1.5	1.9	2.1	5.6	
		CV	4.3	26.1	22.2	21.9	

Met. = metconazole Az. = azoxystrobin
F. = *F. culmorum* *M.* = *M. nivale*

DISCUSSION

In 1998 there was no evidence ($P = 0.05$) from the field trial that fungicide treatment gave control of FEB. This was not unexpected, as control in the field is known to be poor and usually inconsistent (Liggitt, 1997). Late harvesting of the grain owing to prolonged wet weather, may in part have caused the low seed viability.

Seedling emergence in 1998 was low. This was caused in part by the very poor seedbed conditions and water logging following drilling. However, the use of a seed treatment did give a significant improvement in the percentage emergence over untreated seed, and the significant ($P < 0.05$) differences between the ear sprays and seedling emergence although small, were interesting.

There was evidence from the 1999 field trial results that some control of FEB was possible in the field, but it was limited by the species of pathogen present. If both pathogens were present then the azoxystrobin + metconazole mixture proved the most effective.

The reason that fungicidal control of FEB in the field is limited may be explained by the seed infection data for the two pathogens used in this study. When *M. nivale* seed infection was reduced by treatment with azoxystrobin an increase in *F. culmorum* was observed. Control of the disease symptoms caused by *M. nivale* may have occurred in the field and been masked by an increase in those caused by *F. culmorum*. Azoxystrobin is reported to be more active against *M. nivale* than *F. culmorum* so the lack of control is not unexpected. However, the increase in *F. culmorum* seed infection following application of azoxystrobin inoculated with the pathogen mixture is interesting. The increase in *F. culmorum* infection may have resulted in a change in the ear microflora following the fungicide treatment (Liggitt, 1997). There is evidence from the 1998 field trial that the balance between the saprophytes and pathogens present on the ear may affect the severity of FEB symptoms. Azoxystrobin may remove many saprophytes and some pathogens i.e. *M. nivale* from the ear leaving *F. culmorum* to colonise the ear and infect the seed with little competitive opposition.

As would be expected, seed treatment with fludioxonil increased seedling emergence. The fungicide used on the ear also affected emergence, and where azoxystrobin controlled *M. nivale* infection, seedling emergence was greatest. Overall, these results indicated that the use of fludioxonil seed treatment gave a benefit with respect to seedling emergence, and that the benefit afforded by seed treatment was significantly greater when metconazole was used on the ear. When azoxystrobin was used to control *M. nivale* inoculum, no significant benefit from seed treatment was seen. Interpretation of these results with respect to a commercial situation is difficult. It is unlikely that the species infecting the ears of a crop will be known, therefore given the complex nature of this disease its potential to infect seed will not be clear, and the choice of fungicide to use even less so. A sensible approach may be to use a mixture of azoxystrobin and metconazole in an attempt to reduce seed infection and then base the decision to use a seed treatment on the results of a seed infection test.

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Fludioxonil, a low use rate seed treatment for the control of *Fusarium* on corn and potatoes

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ABSTRACT

Fludioxonil has worldwide registration on major food crops such as cereals, corn, sorghum, potato, legume vegetables, and rice. It belongs to the phenylpyrrole class of chemistry and has a unique mode of action. Fludioxonil has demonstrated activity against several species of *Fusarium* that cause damping-off in corn, including *F. moniliforme*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *and F. subglutinans*. Laboratory studies with the listed *Fusarium* spp. show fludioxonil significantly increased seed germination by a mean of 52.1% compared to the standard captan. On inbred corn fludioxonil significantly increased field emergence compared to captan. Colonization of the seed was reduced by 43.9%, compared to captan. In trials on 9 varieties of potatoes fludioxonil showed suppression of the decay caused by *Fusarium sambucinum* on seed pieces, the level of suppression was superior to that given by thiophanate-methyl and mancozeb.

INTRODUCTION

Fludioxonil is a novel molecule with fungicidal activity that has, over the past 6 years, received worldwide registration on major food crops such as cereals, corn, sorghum, potato, legume vegetables, and rice. Ciba-Geigy Ltd. (now Syngenta Crop Protection) discovered fludioxonil as a result of a molecule optimisation process using a natural product, pyrrolnitrin, as a lead structure. Pyrrolnitrin is an antifungal secondary metabolite first isolated from *Pseudomonas pyrocinia* in 1965. Fludioxonil has a broader range of activity, improved activity against key fungi, and increased light stability compared to pyrrolnitrin. Biochemical studies indicate that the mode of action of fludioxonil can be described as the inhibition of a protein kinase (potentially) involved in the osmosensing signal transduction pathway. Cell water uptake, the cell membrane transport process and cell wall synthesis are all affected and cell death occurs. This mode of action is unique to the phenylpyrroles (Pillonel & Meyer, 1997). There is no known cross resistance to other fungicides such as vinclozolin, cyprodinil and carbendazim.

Fludioxonil is a contact fungicide when used as a seed treatment. For some types of seed, and under different environmental conditions, there can be limited uptake into the seed and seedling. However, chemodynamic studies with fludioxonil applied as a seed treatment on wheat indicate that a small proportion, approximately 4%, of the applied amount of the active ingredient does move into the seed and thence to the coleoptile. This does not represent the classic movement associated with systemic compounds. The even smaller amount that reaches the leaves is quickly degraded.

Fludioxonil has activity against a range of major, historically difficult to control pathogens, including *Fusarium* spp., *Rhizoctonia* spp. *Sclerotinia* spp. *Tilletia*, and *Helminthosporium solani*. Within this range of fungi, it is active against several different types of diseases including seed rots, pre- and post-emergent damping-off, tuber rot and root rots. It also has activity against common bunt (*Tilletia caries*) in wheat.

METHODS AND MATERIALS

Studies were carried out by Iowa State University with six species of *Fusarium* that cause damping-off of corn. These studies were conducted by placing 10 treated corn seed on agar plates inoculated with *Fusarium* spores, there were three replications. The plates were incubated for 14 days at 15°C. Treatment rates for captan and fludioxonil were 62.5 and 2.5 a.i./100 kg, respectively. The rates reflect those used by the corn seed producers in the U.S.

Inbred corn field studies were conducted in 1997 by The Tryon Group, Inc., Madison, WI., an independent seed treatment research company. These studies, included seed with common inbred corn genetic backgrounds from major seed companies in the U.S. Trials were conducted comparing fifteen different in-bred corn lines at five different locations in the central Corn Belt of the United States for a total of 150 individual plot comparisons. Fungicides were applied at the following commercial field use rates; fludioxonil + metalaxyl (Apron FL) 2.5+2g a.i./100kg and captan (Captan 400) + metalaxyl 55+2 g a.i./100 kg of seed respectively. All treatments were replicated twice at each location and percent total field emergence was evaluated by plant stand counts for each treatment.

RESULTS

The results of the studies conducted by Iowa State University (Munkvold, 1998) with six species of *Fusarium* that cause damping-off in corn shows how the fludioxonil-treated corn seed resulted in higher seed germination (Table 1) and lower numbers of seed colonized by the fungus than those treated with captan, (Table 2).

The inbred corn field studies conducted by the Tryon Group show improved inbred corn field emergence with fludioxonil-treated seed when compared to captan-treated seed.

Results from these trials are shown graphically, where every fludioxonil field emergence data point is plotted against the corresponding captan field emergence point for each respective inbred and field plot location. (Figure 1) Data points above the captan emergence isoline show increased fludioxonil field emergence collectively over all locations and all inbreds tested. Data points below the captan emergence isoline collectively show decreased fludioxonil field emergence.

Inspection of the plot indicates more weight (data points) above the line than below indicating higher overall percent emergence for fludionoxil. The results of a t-test performed on the data also indicate a significant difference in favor of the fludioxonil treatment group ($p = 0.005$) for percent emergence.

Table 1. Effects of seed treatments and *Fusarium* spp. on germination of corn seeds after 2 weeks at 15°C. Data are the number of germinated seeds out of ten; means of two experiments.

	Number of seeds germinated		
Fusarium species	Captan	Fludioxonil	Untreated
F. moniliforme	3.8	6.6	8.2
F. moniliforme	5.0	6.4	7.8
F. graminearum	2.4	7.2	5.2
F.graminearum	2.4	6.8	5.4
F. oxysporum	6.4	7.8	8.0
F. proliferatum	5.6	6.4	8.4
F. proliferatum	4.6	5.6	7.2
F. solani	4.3	8.7	6.0
F. subglutinans	5.4	7.8	6.6
F. subglutinans	5.0	8.4	5.6
None	7.8	9.0	9.2
Mean	4.8c[1]	7.3a	7.1a

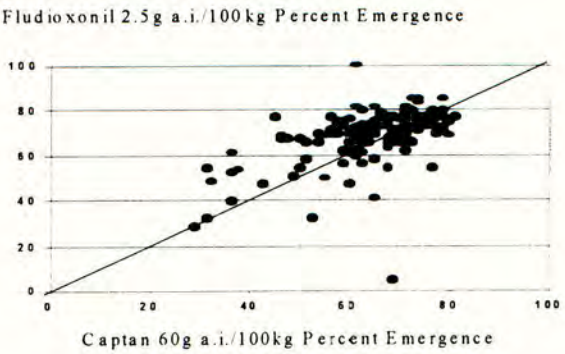
[1]P<0.05. Values followed by the same letter are not significantly different.

Table 2. Effects of seed treatments and *Fusarium* spp. on colonization of corn seeds at 15°C. Data are the number of colonized seeds out of ten; means of two experiments.

	Number of seeds colonized		
Fusarium species	Captan	Fludioxonil	Untreated
F. moniliforme	10.0	6.4	10.0
F. moniliforme	10.0	5.8	10.0
F. graminearum	10.0	5.8	10.0
F.graminearum	10.0	5.4	10.0
F. oxysporum	10.0	3.6	7.2
F. proliferatum	10.0	7.2	10.0
F. proliferatum	10.0	7.8	10.0
F. solani	9.3	6.3	10.0
F. subglutinans	10.0	4.2	10.0
F. subglutinans	10.0	3.6	10.0
None	8.8*	4.6*	6.4*
Mean	9.8a[1]	5.5b	9.4a

**Penicillium* spp.
[1]P<0.05. Values followed by the same letter are not significantly different.

Figure 1. Comparison of fludioxonil vs. captan on field emergence of inbred corn, trials conducted in five Midwestern U.S. states by Tryon Group. All seed received metalaxyl at 2g a.i./100kg.



In potatoes, fludioxonil is active against seed piece decay caused by *Fusarium sambucinum*, and *F. solani var. coeruleum*, the two most important species involved with this disease. A recent study reported from the University of Idaho (Nolte, 2000). suggests that fludioxonil seed treatment on potato provided consistent suppression of Fusarium seed piece decay caused by *F. sambucinum* across 9 different potato varieties (Table 3).

Table 3. Percent incidence of seed decay in different potato varieties due to infection of *Fusarium sambucinum*.

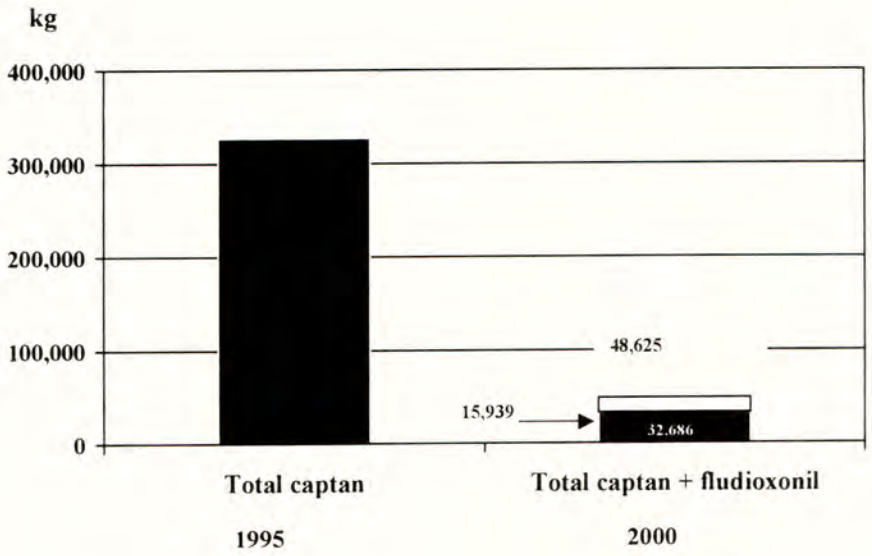
Treatment	g ai/ 100 kg	Russet Burbank (34)[1]	Bannock (1)	Gem Russet (1)	Ida-Rose (1)	Nor-donna (1)	Ranger Russet (4)	Umatill (3)	Russet Norkotah (3)	New Leaf (2)	Total of 50 Lots[2]
Untreated	-	32.1	22	0	66	6.0	30.5	39.2	30	19	30
Thiophanate-methyl	25	4.8	1	1	23	6.6	13.5	24.3	10	2.5	0.1
Mancozeb	30	0.0	1	0	12	0.0	0.5	0.0	0	1.0	0.01
Fludioxonil	2.5	0.1	0	0	0	0.0	0.2	0.3	0	0.1	0.0
LSD (0.05)		0.01	0.06	0.01	0.1	0.03	0.04	0.05	0.07	0.04	0.01

[1] Number in parenthesis is total number of seed lots tested under each variety.

[2] The incidence of the 50 seed lots was conducted with a pooled data of 19,920 observations using general linear model procedure.

Thiophanate methyl = Tops®
Fludioxonil = Maxim®

Figure 2. Estimated decrease of seed treatment fungicide use in corn with introduction of fludioxonil.



In the U.S.A. approximately 90% of the corn seed received the combination of fludioxonil and mefenoxam in 2000. The USDA estimate of corn acres planted in 2000 was 31.2 million hectares. The market share estimate was derived from Syngenta sales and product shipping records of these products to corn seed producers for the period. This has reduced the total pesticide load to the environment by 85.1% since 1995 when Captan was planted on nearly 100% of the USDA estimate of 29.2 million hectares. (Figure 2)

DISCUSSION

Fludioxonil also controls several AG groups of *Rhizoctonia solani*. On radish, in vitro activity at low rates was demonstrated against AG 1-1B, AG 1-1C, AG 2-1, AG2-2 and AG 2-IIIB on radish (Colburn & Miller, 1999). Work is proceeding to determine the activity of fludioxonil on potatoes

Fludioxonil is frequently combined with mefenoxam seed treatment because fludioxonil has no activity against Oomycetes such as *Pythium* or *Phytophthora*. Both of these Oomycetes can cause significant loss of stand and yield in several areas of the globe if their control is not addressed.

Fludioxonil has been widely adopted by growers and major seed producers worldwide. The rapid adoption is due to improved efficacy over standard products, the favorable human and environmental safety profile, improved occupational exposure profile, low application rates (2.5 to 5 g a.i./100 kg seed), worldwide registration, excellent crop and seed storage safety, and improved handling in seed plants compared to the standards that have been used over the past 20-30 years.

Fludioxonil is registered in 42 countries as a seed treatment. Products containing fludioxonil are sold as several trade names across the world, including Influx® on maize and Celest® on wheat in France, Maxim® XL in the major maize producing countries, and Beret Gold® in the UK and Netherland for cereals.

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Control of soil-borne common bunt (*Tilletia tritici*) by seed treatment

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ABSTRACT

Infections from the seed are the most important contribution to an infection of common bunt (*Tilletia tritici*) but spores in the soil can also serve as primary inoculum and give rise to new and unexpected attacks. Although low levels of infection may occur in the first year of planting untreated wheat seed, this can be a way to maintain common bunt in the field. Normally new fungicide seed treatments are tested for their efficacy against the seed-borne infection. However, with the increasing awareness of soil-borne common bunt it is important that the seed treatments also have a high efficacy against soil-borne infections. In Denmark the products are now tested on a routine basis, on both seed- and soil-borne infection. The results of trials from 1995-1998 show very good control of both seed- and soil-borne infections with bitertanol + fuberidazole. Low levels of control of soil-borne infection were obtained with guazatine, and with the biological product *Pseudomonas chlororaphis*. Seed treatments with bitertanol + fuberidazole or difenoconazole are now approved in Denmark for control of soil-borne common bunt.

INTRODUCTION

Normally common bunt (*Tilletia tritici, syn. T. caries*) infects the wheat plant from spores present on the seed but spores in the soil can also infect the wheat via the coleoptile (Johnsson, 1990; Nielsen & Nielsen, 1993; Borgen, 2000). The most important contribution to the epidemic is clearly through the seeds but soil-borne spores can give rise to low and unexpected attacks in the field. If wheat is grown continuously or in short rotations these low incidences can, over few years, lead to severe infections (Borgen, 2000). Normally seed treatments are tested for their efficacy against the seed-borne phase but it is also important to ensure that the products have a high efficacy against soil-borne infections. There have been previous reports on evaluation of seed treatments on soil-borne bunt (Holton *et al.*, 1954; Hoffmann & Waldher, 1981; Wainwright & Morris, 1989) but no report on the modern seed treatment products used to-day in Denmark.

In Denmark new products for seed treatment are tested in field trials, at the Danish Institute of Agricultural Sciences, as a part of the biological approval scheme. For wheat we use seed artificially inoculated with common bunt as a standard (Nielsen & Jørgensen, 1994). When we became aware of the risk of soil-borne infection we developed a simple system where spores of common bunt are placed in the soil before sowing. Several methods have previously been used for testing the efficacy of seed treatments on soil-borne common bunt. Hoffmann & Waldher (1981) sprinkled a water suspension of common bunt spores at 0.5 g of teliospores per 1.5 m row in the opened furrows, just before sowing. This seems to have been a procedure used also in some early USA work on testing soil-borne infections, e.g. Holton *et al.* (1954). Wainwright

& Morris (1989) incorporated, during sowing, a mixture of 1 g spores with 100 g of sand per plot.

Seed treatments currently approved for the control of seed- and soil-borne common bunt in Denmark are listed in Table 1. Criteria for approval is 99-100% control of seed-borne infections and 97-100% of soil-borne infections.

Table 1. Seed treatments approved by Danish Institute of Plant and Soil Sciences for the control of common bunt (*Tilletia tritici*), seedling blight (*Septoria nodorum*) and *Fusarium spp.* in wheat in Denmark.

Product	Active ingredients	g a.i./l	Dose ml/ 100 kg	Seed [1)]	Soil
Sibutol LS 280	bitertanol+fuberidazole	280+18	100	+	+
Celeste 025 FS	fludioxonil	25	200	+	
Dividend 37,5 FS	difenoconazole	37.5	200	+	+
Beret FS 050	fenpiclonil	50	200	+	
Beret Combi	fenpiclonil+difenoconazole	50+50	200	+	

1) Approved for control of seed-borne (Seed) and soil-borne (Soil) infection.

MATERIALS AND METHODS

The field trials were designed to test winter wheat with different treatments in complete randomised blocks and 4 replicates. Plot size in all experiments was single 9 m rows with 400 seeds. The seeds were sown with a standard single row seed drill at 3 cm depth. In the experiments to control soil-borne common bunt refered to in Table 2 either 3 g or 12 g, of a mixture of 15 g bunt spores in 450 g of sand, were placed in the furrow just before sowing. In the trial carried out in 1998 refered to in Table 3, 60 g of the spore/sand mixture was used per 9 m row. This is now the standard method of inoculation for trials for the control of soil-borne common bunt. In the trials to evaluate seed-borne infections, the seeds were artificially inoculated, before seed treatment, with either 0.25 g or 5 g spores of *Tilletia tritici* per kg wheat. Field emergence was counted at GS 10 and at GS 85 the number of plants infected with common bunt was counted.

The active ingredients and the rates of use of the products used in the trials are shown in Table 1. In the trials refered to in Table 3 products also used were maneb 140 g a.i./100 kg (used as DLG Maneb: maneb 700 g a.i./kg), guazatine 60 g a.i./100 kg (used as Panoctine 300 g a.i/l) and *Pseudomonas chlororaphis* 600 ml spore suspension/100 kg (used as a MA 342).

RESULTS

In Table 2 results are shown from trials with two levels of soil-borne inoculation compared with two levels of seed-borne inoculation. Spores placed in the soil before sowing generally gave moderate levels of infection although less than that obtained after seed 5 g spores inoculation. The results of the 1995-1998 trials show very good effects against soil- and seed borne infections with both rates of bitertanol + fuberidazole. Efficacy was complete against the seed-borne infections and of the order 97% against the soil-borne infection over the four year period ranging from a mean of 91% (1996) to 99.8% (1998).

Table 2. Control of seed- and soil-borne common bunt (*Tilletia tritici*) in winter wheat, 1995-1998.

Treat-ments	Dose g a.i./ 100 kg	Seed inf. g spores per kg wheat	Soil inf. g spores per m row	% plants with common bunt							
				1995	1995	1996	1996	1997	1998	1998	Mean 7 trials
Untreated		0	0	1.0cd	2.2e	0.2d	7.8c	4.1c	0.0d	4.7c	2.9d
Untreated		0	3	2.2c	4.8d	16.0b	23.4b	15.4b	27.7b	22.8b	16.0c
Bit.+fub.	28+1.8	0	3	0.4d	0.6f	0.1d	2.1d	0.0d	0.1d	0.0d	0.5e
Bit.+fub.	56+3.6	0	3	0.1d	0.2f	0.6d	1.7d	0.2d	0.0d	0.0d	0.4e
Untreated		0	12	4.0b	8.1c	13.9c	23.9b	18.3a	29.6b	33.8a	18.8b
Bit.+fub.	28+1.8	0	12	1.1cd	0.7ef	0.5d	1.5d	0.1d	0.1d	0.0d	0.6e
Bit.+fub.	36+3.6	0	12	0.6d	0.9ef	0.1d	2.8d	0.0d	0.1d	0.0d	0.6e
Untreated		0.25	0	4.6b	11.2b	19.5a	26.0ab	19.0a	24.6c	4.0c	15.6c
Untreated		5.0	0	27.0a	74.3a	19.6a	28.7a	20.3a	36.1a	24.5b	32.9a
Bit.+fub.	28+1.8	0.25	0	0.2d	0.0f	0.1d	0.0d	0.0d	0.0d	0.0d	0.0e
Bit.+fub.	28+1.8	5.0	0	0.0d	0.0f	0.0d	0.0d	0.0d	0.0d	0.0d	0.0e

Bit.+fub.=Bitertanol + fuberidazole. Uninoc.=Uninoculated. Figures in columns with the same letter are not significantly different (P 0.05).

The low level of infection in the untreated seed and uninoculated soil plots (0.2-7.8%), and also in the 1998-trials (Table 3) presumably came from spores in the soil remaining from trials with common bunt in previous years. Products like guazatine, maneb and biological products with *Pseudomonas chlororaphis* gave only a small effect on soil-borne infections (Table 3).

Table 3. Control of seed- and soil-borne common bunt (*Tilletia tritici)* in winter wheat, 4 trials, 1998.

Treatments	Dose g a.i./ 100 kg	% plants with common bunt			
		Seed 0.25 g/kg	Seed 5 g/kg	Seed 5 g/kg	Soil Inoculation[1)]
Untreated/uninoculated		3.1	3.8f	8.8g	4.2d
Untreated/inoculated		17.1	38.2a	45.1a	25.0bc
Guazatine	60	3.1	7.9de	15.8de	21.1c
Bitertanol +	2.8	0	0g	0i	0d
fuberidazole	1.8				
Maneb	140	0.7	5.2ef	8gh	23.9bc
P. chlororaphis	600[2)]	2.5	17.4b	25.7c	26.7bc

[1)] See materials and methods. Figures in columns with the same letter are not significantly different (P 0.05). [2)] ml Bacteria suspension.

DISCUSSION

Over a period of four years from 1995-1998 trial results showed very good effects against soil-borne bunt infections with bitertanol + fuberidazole. Two dose levels were included in the trials to see if a high dose was necessary to control soil-borne infections. The results show that the standard dose was sufficient. Other trials (Nielsen, 1998) have shown good effects with difenoconazole, and triticonazole + iprodione. The products have been given a biological approval for controlling seed- and soil-borne common bunt. Interestingly low levels of control of soil-borne infection were obtained with guazatine. In our trials with biological products the effect against soil-borne bunt was, as expected, very low.

In a wheat field where there has been an attack of common bunt there is a high risk of infection if wheat is sown again in the autumn, especially under dry conditions. In the following year(s) there may still be a risk of infections in the field although at a lower level. This presents a risk not only in conventional agriculture but also, and especially, in an organic farming system. Once common bunt is first introduced on the farm it can be very difficult to control.

The soil-borne phase can be a way to sustain low levels of the pathogen and to be the starting point of a new epidemic. Thus it is very important that the soil-borne phase is controlled effectively and as early as possible. The seed treatments used for controlling common bunt should have a high efficacy on both phases of the pathogen.

The trials have shown good results and with the seed treatments available on the market today, it is possible to choose products with a high efficacy both against seed- and soil-borne common bunt.

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Control of *Microdochium nivale* with fludioxonil seed treatments

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ABSTRACT

Microdochium nivale is the most important and widespread pathogenic fungus causing damping-off and reduced establishment in cereals in northern Europe. Since their introduction as seed treatments over ten years ago, the phenylpyrroles (fenpiclonil and fludioxonil) have become the standard for protecting cereals against damage from *M. nivale* during crop establishment and beyond. Trial results from Switzerland, Germany and UK compare the performance of fludioxonil with other commercially available seed treatments. In all these experiments, fludioxonil has given effective control of *M. nivale* and shown outstanding duration of control against post-emergence damping-off and stem browning symptoms.

INTRODUCTION

Fusarium seedling blight of cereals, caused by *Microdochium nivale* and *Fusarium* spp., results in reduced germination, seedling damping-off and stem browning. Under UK conditions, *M. nivale* is the most important of these pathogens. Cockerell and Rennie (1996) reported 99% of seed samples to be infected in 1992 and 1993. Reeves and Wray (1994) found over 90% of seed samples to be infected and over 90% of these failed to meet the advisory limit of 5% seed infection. In continental Europe such survey data are scarce but the ease with which *M. nivale* infected seed can be located for use in field trials and the considerable problems of snow mould in Germany and Switzerland, suggest that the problems associated with this pathogen are on a similar scale to those of the UK. There is also evidence of the importance of *M. nivale* during the later stages of crop growth. A UK survey of stem base disease showed 41% of crop infected with *M. nivale* in 1997 and 97% in 1998 (Bardsley *et al*, 1998).

The phenylpyrroles have been used as seed treatments in cereals for control of Fusarium and other seed-borne diseases for over ten years (Koch and Leadbeater, 1992). The first seed treatment from this chemical family, fenpiclonil, has now been replaced by fludioxonil, trade names Beret Gold® and Celest®, which has the advantage of a very low rate of active ingredient in addition to its broad spectrum control of seed-borne diseases (Leadbeater et al, 1990). Results are presented here to demonstrate the effectiveness of fludioxonil in protecting the emerging crop from seedling blight and also the lasting activity in preventing subsequent infections of stem-base browning and nodal infection. The activity of fludioxonil is compared to other commercially available seed treatments.

MATERIALS AND METHODS

Field trials were of randomised complete block design with four replicates and a minimum plot size of $10m^2$. Trials used seed naturally infected with *M. nivale* and were drilled using a small plot drill. Both fludioxonil and commercially available seed treatments were applied using a small batch seed treater such as the Hege 11 or Mini-Rotostat. Crop establishment was assessed by counting the number of plants per 1m row at five locations per plot and at least 25 plants per plot were sampled for Fusarium disease assessments. Plants were scored for presence or absence of disease. In some trials a disease index was calculated based on the severity of infection where plants were put into four different classes. The numbers of plants in these classes were then used as the basis for the index calculation. The formula for the index calculation was:

Disease Index = (n1 * 0) + (n2 * 25) + (n3 * 50) + (n4 * 100)/(n1+n2+n3+n4)

n1 = number of plants in class 1 (no infection), n2 = number of plants in class 2 (low infection), n3 = number of plants in class 3 (medium infection), n4 = number of plants in class 4 (high infection).

A controlled environment growth room experiment was carried out by Syngenta, Switzerland. The trial was of a randomised complete block design with three replicates. One hundred seeds per plot were planted in trays of loam and maintained at 3°C for 30 days in the dark and then at 10-12°C, with 12 hours of light per day for the duration of the experiment. The total number of emerged plants and those exhibiting disease symptoms were counted at emergence and intervals following this.

Statistical analysis compared treatment means using a series of one-sided orthogonal contrasts each performed, after appropriate data transformations, at a 5% significance level. The quoted treatment results were obtained as part of larger field trials with more treatments. The contrasts are indicated in each table and statistical significance is denoted [✓] with no significant difference [✖].

RESULTS

Table 1: Control of *M. nivale* in winter wheat: growth room experiment conducted in Switzerland 1999 (100 seeds planted).

Treatment	Formulation type and concentration g a.i./l	Dose g a.i./100kg seed	Emergence count	Final stand count	% healthy seedlings
1 Untreated			29.7	42.7	33.0
2 Triticonazole	FS 25	5	51.3	61.0	33.0
3 Tebuconazole	FS 60	2	48.0	62.0	32.7
4 Fluquinconazole	FS 167	75	35.7	47.3	27.3
5 Fludioxonil	FS 25	5	81.3	88.7	83.3
Days after planting (DAP)			34	39	74
Significance 2,3,4,5 compared to 1			✓	✓	Not determined
Significance 5 compared to 2,3,4			✓	✓	✓
Transformation			arcsine	arcsine	arcsine

cv Bernina 49% seeds infected with *M. nivale*.

A growth room experiment to compare the activity of fludioxonil and triazole seed treatments was conducted under controlled conditions (Table 1). This methodology has been developed by Syngenta and has proven to be a reliable means of testing the activity of seed treatments against *M. nivale* with good correlation to field performance. The initial emergence count and the final stand count data showed that the triazole based treatments increased the number of plants compared to untreated but fludioxonil further increased the number of seedlings. A measure of lasting effect is provided by the % healthy seedlings results which indicate that the triazoles lack persistence of activity compared to fludioxonil.

Field trial data from Germany showed all treatments to significantly increase the number of healthy plants and decrease the disease index (Table 2). In both trials the final disease index was higher for bitertanol + fuberidazole treated seedlings than those treated with carboxin + prochloraz or fludioxonil + tebuconazole.

Table 2: Control of *M. nivale* in winter wheat: field trials conducted in Germany 1995.

	Treatment		Formulation type and concentration g a.i./l	Dose g a.i./100kg seed	Stand count		Disease index	
					Trial 1	Trial 2	Trial 1	Trial 2
1	Untreated				9.5	24.3	36.3	38.8
2	Carboxin prochloraz	+	LS 400 + 80	80 + 16	50.5	49.0	10.9	10.6
3	Bitertanol fuberidazole	+	FS 375 + 23	75 + 4.6	19.5	40.8	27.6	19.9
4	Fludioxonil tebuconazole	+	FS 25 + 5	5 + 1	52.5	47.8	5.4	11.6
DAP					141	53	141	53
Significance 2,3,4 compared to 1					✓	✓	✓	✓
Significance 3 compared to 2,4					✓	✓	✓	✓
Significance 4 compared to 2					✖	✖	✓	✖
Transformation					None	None	arcsine	arcsine

Comparison of the performance of guazatine + tebuconazole, fludioxonil + tebuconazole and fludioxonil, showed all treatments to significantly decrease the number of infected plants (Table 3).

Table 3: Control of *M. nivale* in winter wheat: field trial conducted in Germany 1996.

	Treatment		Formulation type and concentration g a.i./l	Dose g a.i./100kg seed	Stand count	% infected plants	Disease index
1	Untreated				24.5	49.5	18.3
2	Guazatine tebuconazole	+	FS 300 + 15	60 + 3	42.2	8.0	2.1
3	Fludioxonil		FS 25	5	41.5	10.0	2.8
4	Fludioxonil tebuconazole	+	FS 25 + 5	5 + 1	39.8	2.0	0.8
DAP					28	182	182
Significance 2,3,4 compared to 1					✓	✓	✓
Significance 4 compared to 2,3					✖	✖	✓
Transformation					None	arcsine	arcsine

The disease index however showed a clear advantage for fludioxonil + tebuconazole. The data presented in Table 1 show that whilst tebuconazole increased the number of plants emerging, it had no effect on the incidence of disease on emerged plants compared to untreated seeds. However it is apparent from the data in Table 3 that the addition of tebuconazole, at 50% of the rate tested in the growth room experiment, added to the control provided by fludioxonil alone. Fludioxonil alone reduced the disease index by 85% and fludioxonil + tebuconazole by 96%.

The performance of guazatine and fludioxonil was compared in a series of trials carried out in Scotland between 1992 and 1995 (Table 4). In all trials, both treatments gave a large increase in the number of plants with the mean data showing an increase of 193% over untreated for fludioxonil and 177% for guazatine.

Table 4: Control of *M. nivale* in winter wheat: field trials conducted in Scotland (1992, 94 & 95, 1 trial per year) — crop establishment. From Burke *et al.* (1996).

Treatment	Formulation type and concentration g a.i./l	Dose g a.i./100kg seed	No. plants per m row			
			1992	1994	1995	Mean
1 Untreated			20.5	25.4	16.6	20.8
2 Fludioxonil	FS 025	5	50.0	42.8	27.9	40.2
3 Guazatine	LS 300	60	44.4	39.4	26.5	36.8
DAP			37	104	53	
Significance 2,3 compared to 1			✓	✓	✓	
Significance 3 compared to 2			✖	✓	✓	
Transformation			None	None	None	

1992 cv Riband 70%; 1994 cv Haven 58%; 1995 cv Hunter 34% seeds infected with *M. nivale*.

Further comparisons between fludioxonil and guazatine were made in trials conducted in eastern England and eastern Scotland in 1993 (Table 5). Stem-base browning symptoms caused by *M. nivale* were significantly reduced by guazatine (61.6% control) but this was less effective than fludioxonil (81.1% control). This result confirms the outstanding persistence of control of *M. nivale* provided by fludioxonil.

Table 5: Control of *M. nivale* in winter wheat: field trials conducted in UK (1992-3) - % control of stem-base browning (mean of 6 trials).

Treatment	Formulation type and concentration g a.i./l	Dose g a.i./100kg seed	% Control of stem-base browning 109-208 DAP (% plants infected in untreated)
Untreated			(60.2%)
Fludioxonil	FS 25	5	81.8
Guazatine	LS 300	60	61.6

cvs Riband 70% and Slejpner 71% seeds infected with *M. nivale*.

Control of nodal Fusarium was observed in one UK trial in 1994 (Table 6). Carboxin + thiabendazole reduced the untreated level of 78% of plants infected by 36% and fludioxonil by 77%.

Table 6: Control of nodal Fusarium in winter wheat: field trial conducted in UK (1994). From Burke *et al.* (1996).

	Treatment	Formulation type and concentration g a.i./l	Dose g a.i./100kg seed	% nodal browning
1	Untreated			78.0
2	Fludioxonil	FS 25	5	20.0
3	Carboxin / thiabendazole	FS 360 + 20	90 + 5	65.0
DAP				176
Significance 2,3 compared to 1				✓
Significance 2 compared to 3				✓
Transformation				arcsine

cv Haven 58% of seeds infected with *M. nivale*.

DISCUSSION

M. nivale is recognised as a serious pathogen of cereals causing problems of establishment as a result of seed-borne infections. The data presented in this paper demonstrate the effectiveness of the phenylpyrrole seed treatment fludioxonil in controlling the disease. Comparison with other active ingredients shows that seed treatments were generally effective in increasing the number of seedlings that emerge from *M. nivale* infected seed. However in addition, fludioxonil demonstrated a greater persistence of activity thereby preventing, or reducing dramatically, seedling and young plant infections.

Yield responses of 1.0t/ha over untreated and 0.4t/ha over non-phenylpyrrole treatments have been observed in winter wheat as a result of this level of disease protection (Burke et al, 1996). This persistence of activity from fludioxonil has also resulted in a significant reduction of nodal Fusarium symptoms. Tillers with nodal infection have been shown to yield 20% less than tillers without such lesions (Anon., 1983). Severe nodal browning may also weaken stems and increase lodging.

To provide a complete spectrum of control of seed-borne diseases, combinations of active ingredients may be required. The combination of fludioxonil with tebuconazole was shown to increase the level of control of *M. nivale* compared to the individual components. In addition this mixture has a broader spectrum of activity compared to fludioxonil used alone, controlling diseases such as dwarf bunt (*Tilletia controversa*) and loose smut (*Ustilago nuda*).

In the future, *M. nivale* looks likely to remain an important disease of cereals. The changing pattern of arable farming with trends towards larger farms and reduced labour means that many growers are experimenting with earlier sowing dates to allow sufficient time to plant their whole farm before the autumn weather deteriorates (Anon., 2000). This practice is often accompanied by the use of much lower seed rates than have traditionally been adopted. Very low seed rates are also practised for growing hybrid cereals. In these scenarios, the importance of each individual seed is increased as seeds are spaced more widely in the row and the ability of the crop to compensate for seeds that fail to establish as healthy seedlings is reduced. Although technologies such as polymerase chain reaction diagnostics are now available to test for seed-borne Fusarium, the endemic nature of *M. nivale* in northern

available to test for seed-borne Fusarium, the endemic nature of *M. nivale* in northern Europe means that in most years, farmers are unlikely to be able to choose seed free from infection. Seed treatments will continue to have a vital role to play in protecting the crop against *M. nivale*. Fludioxonil, with its exceptional level of activity, will continue to be the leading solution for this disease.

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Pathogenesis related proteins induced in wheat following seed treatment with carboxin

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ABSTRACT

Carboxin is a systemic anilide fungicide used as a seed treatment for the control of loose smut and other diseases on several crops, including wheat. It has been observed that carboxin seed treatment often induces in wheat a general healthy growth and promotes higher yields. This aspect prompted us to explore the possible physiological mechanisms, such as pathogenesis related protein accumulation, possibly activated during the early germination of wheat seed treated with carboxin.

Wheat seedlings (cultivar S.Pastore) showed a decrease in growth development during early germination; however, no differences were recorded during later stages. The protein pattern analysis of seedlings during the early germination stages revealed a differential induction of β 1,3 glucanase and chitinase isoenzymes after carboxin treatment.
These results suggest that seed treatment with carboxin could facilitate the control of soil-borne pathogens through direct antifungal activity and, potentially, by activating plant defence mechanisms.

INTRODUCTION

Acquired resistance (AR) is an inducible resistance mechanism effective against fungal, bacterial and viral pathogens. AR can be restricted to the tissues treated with the activator (local acquired resistance, LAR) or can be characterised by the induction of long lasting, systemic resistance (systemic acquired resistance, SAR).

In wheat, SAR is thought to be induced by incompatible pathogens and by chemical inducers such as salicylic acid (SA), 2,6 dichloroisonicotinic acid (INA) and benzo(1,2,3) thiodiazole-7-carbothioic acid *S*-methyl ester (BTH) (Görlach *et al.*, 1996; Schaffrath *et al.*, 1997; Molina *et al.*, 1999).

During SAR response, a set of gene families encoding pathogenesis related (PR) proteins have been shown to be activated in different plant species, following distinct signalling pathways (Dong, 1998). These proteins are believed to play a central role during the onset and maintenance of SAR.

Carboxin is a systemic fungicide used as a seed treatment, often in combination with thiram, for the control of smut and other diseases on many crops, including wheat. It has been shown that carboxin generally increases emergence, uniformity of seedling size and promotes disease control. In the U.S.A., carboxin is patented as a growth regulator as well as a fungicide.

In the present study we explored the differential induction of β1,3-glucanase and chitinase isoenzymes in protein extracts from wheat seedlings, following seed treatment with carboxin, as a consequence of a possible resistance mechanism activation.

MATERIALS AND METHODS

Plant materials

Bread wheat (cv. S. Pastore) seeds treated with carboxin (analytical standard, Uniroyal Chemical Ltd, Evesham, UK) at a rate of 60 g a.i./100kg were germinated in water agar (1.2% w/v) at 21°C in the dark. Proteins were extracted from seedlings after 24, 48, 72 and 96 hours.

Protein extraction

Untreated and carboxin treated seedlings were harvested at the different stages of development and ground to fine powder in a pre-chilled mortar in liquid nitrogen. Chilled extraction buffer (Tris 20 mM buffer, pH 6.8), containing 1% polyvinylpolypyrrolidone was added to the powder (1ml/g fresh weight tissue). Extractions were carried out at 4°C for 60 min with continuous gentle stirring. The buffer extracts were then centrifuged twice at 18000 *g* for 15 min at 4°C and the clear supernatants were subjected to isoelectric focusing.

Isoelectric focusing of soluble proteins

Isoenzyme separation by isoelectric focusing (IEF) was performed horizontally on Multiphor II apparatus (Amersham Pharmacia Biotech) by using 0.4 mm thick polyacrylayamide gels containing 5% (v/v) ampholytes (Amersham Pharmacia Biotech) covering the pH range 3.5-10.0. The run was carried out at a constant power of 5W for approximately 1.5 h.

Detection of enzymatic defence proteins isoenzymes after IEF

β1,3-glucanase: the gels were washed with water and soaked for 10 min in 50mM sodium acetate pH 5.2, then incubated for 45 min at 40°C with 0.5% (w/v) laminarin (Sigma-Aldrich) in 50mM sodium acetate buffer. After washing with water, the gels were soaked in 0.15% (w/v) 2,3,5-triphenyltetrazolium chloride in 1M sodium hydroxide at 100°C until the bands appeared red (Pan *et al.*, 1989).

Chitinase: the gels were equilibrated in 0.1M sodium acetate buffer, pH 5.2 in a reciprocal shaker for 10 min, then covered with 7.5% polyacrylamide–chitin overlay gels containing 0.04 glycol chitin in 0.1M sodium acetate buffer pH 5.2; the sandwich gels were incubated at 40°C for 2 hours. After incubation, overlay gels were separated from the sandwich gels and treated with 0.01% Calcofluor White M2R (Sigma-Aldrich) in 0.5M Tris pH 8.8 at room temperature for 10 minutes; chitinase isoenzymes were visualised as dark bands on a UV transilluminator (Trudel and Asselin, 1989).

Estimation of pI values

The pI values of isoenzymes were estimated from a regression equation of standard proteins (Amersham Pharmacia Biotech) versus the distance migrated.

RESULTS

Carboxin reduced wheat seedling growth during early germination (24-48 h) as shown in Figure 1; however, at 72 and 96 h treated plant growth was comparable to that of untreated plants. Similar results were obtained on wheat seedlings, following seed treatment with the resistance activators, such as SA (data not shown).

As shown in Figure 2, several β-1,3-glucanase isoforms were constitutively present in protein extracts from carboxin treated and untreated seedlings. Two basic β1,3-glucanases (pI 8.3 and 8.6) were expressed at 24 h in both treated and untreated seedlings, but were specifically induced at 48 h onwards only in treated seedlings. Several chitinases were present in extracts from treated and untreated seedlings in parallel with the expression of β1,3-glucanases. No differences were detected in the protein extracts from treated and control seedlings; however, the basic isoenzymes were substantially more marked in treated than in untreated seedlings.

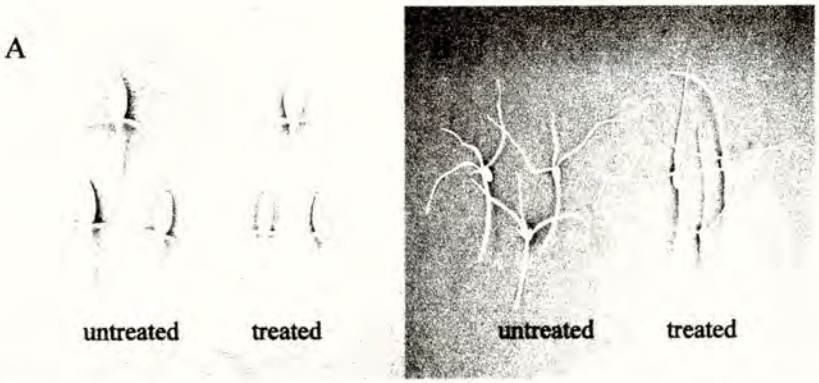

Figure 1. Effect of carboxin on wheat seedling development at 24 h (A) and 96 h after treatment (B).

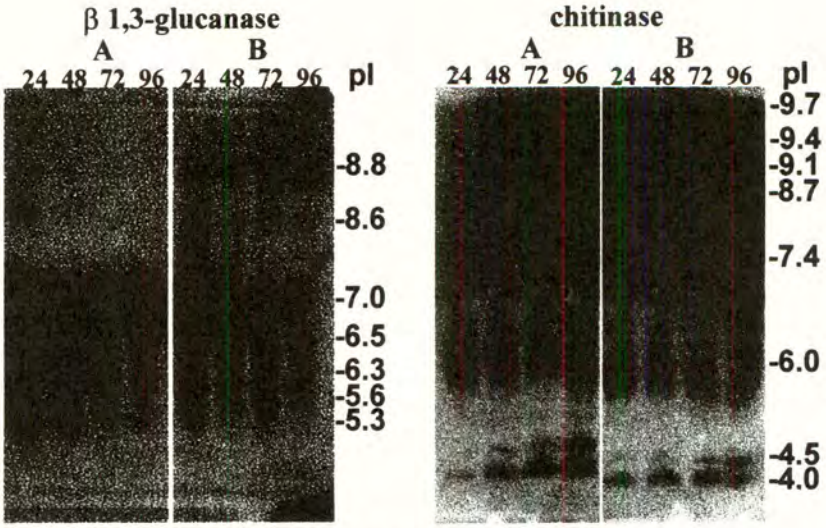

Figure 2. Multiple isoenzymes of β 1,3-glucanase and chitinase separated by isoelectric focusing (IEF) (pH 3.5-10.0) from wheat seedlings. Carboxin treated seedlings (A); untreated seedlings (B). For each enzyme pI values are indicated on the right. Numbers denote hours after treatment.

DISCUSSION

The present study was undertaken to obtain an insight into the induction of PR proteins in germinating wheat in response to seed treatment with carboxin.

Our data indicates that during early germination, two basic β-1,3-glucanases were expressed at 24 h in both treated and untreated seedlings, but at 48 h onwards were specifically induced only in treated seedlings. Moreover, basic chitinase isoenzymes were present at much higher levels in treated than in untreated plants. Since the same amounts of soluble proteins from each protein extract were loaded on the same IEF gel, the intensity of the bands could be considered equal to the specific activities of individual chitinase isoenzymes.

Three basic chitinases and two basic β1,3-glucanases, characterised by estimated pI values similar to those induced by carboxin, have been found to be induced respectively, 72 and 96 h after infection with *F. culmorum* (Caruso *et al.*, 1999). This result indicates that, following seed treatment with carboxin, during germination a common set of PRs are induced in early germination and later upon fungal infection. This set of PRs might be induced by the activation of a common signalling pathway.

Carboxin induced hydrolases may play, in case of fungal attack, both a direct protective role by degrading fungal cell wall components and, an indirect role in the plant defence mechanism by releasing some elicitors from the decaying fungal cell wall that stimulate antifungal compounds accumulation in the host plant (Collinge *et al.*, 1993).

In common with other plant resistance activators, such as SA, carboxin seed treatment produced a transient growth inhibition during early germination of wheat, thus revealing a possible similarity in the metabolic effects. Treatments of different plants with SA or with the more potent synthetic mimics INA and BTH trigger a spectrum of PRs and induce resistance to pathogens (Bostock, 1999).

In wheat, biologically and chemically induced resistance can be established after *Erysiphe graminis* infection or treatment with resistance activators (Schweizer *et al.*, 1989; Kmecl *et al.*, 1995; Görlach *et al.*, 1996); however, the expression of different sets of defence genes has been correlated either with pathogen-induced resistance or chemically-induced resistance (Kmecl *et al.*, 1995; Görlach *et al.*, 1996).

It was reported that these sets of genes are activated by different signalling pathways and that the behaviour of these genes is different from that of SAR genes characterised in dicotyledonous plants (Schaffrath *et al.*, 1997). Moreover, wheat genes encoding two types of PR1 proteins were found to be pathogen-inducible, but not to respond to chemical SAR activators (Molina *et al.*, 1999).

Following carboxin treatment the induction of plant defence proteins, such as PR proteins, in tissues of germinating seeds may play an effect in reducing colonisation of seed- and soil-borne pathogens potentially, by activating plant defence mechanisms. It will be interesting to determine a possible differential induction of other PRs following seed treatment with carboxin, as well as the assessment of induced resistance against wheat pathogens.

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Improved compatibility of metalaxyl-M + fludioxonil seed treatment fungicide with Rhizobium in soya bean production.

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ABSTRACT

The compatibility of seed treatment fungicides and rhizobia is an important requirement for the soya bean crop. Quantitative assays and field studies with a seed treatment formulation of metalaxyl-M + fludioxonil, applied as a tank-mix with a commercially available rhizobium liquid inoculant, has shown high level of compatibility and performance in soya beans. The inoculant tank-mix applied as a slurry with a ready-to-use formulation of metalaxyl-M + fludioxonil fungicide provided 30+% higher survival rate of the rhizobium than when the inoculant was applied independently. The new formulation provided 12.2 nodules per plant compared to 3.0 nodules for the original formulation. This improvement now offers an advantage over existing practices in soya bean seed treatments.

INTRODUCTION

In the current soya bean production system the inoculation of rhizobium on the seed is important for improved fixation of nitrogen in the rhizosphere. Where such inoculation is properly carried out, rhizobia demonstrate good soil colonization and subsequent nodulation in the plant roots. At the same time the use of fungicide seed treatments has shown a high level of success in soya bean against plant pathogenic fungi that undermine seed germination, crop establishment and eventual yield. However, these seed treatments have been found to be detrimental to rhizobia that are inoculated on to the seed. Of the recent seed treatment introductions a formulated ready to apply seed treatment product containing metalaxyl-M + fludioxonil has demonstrated superior control of several key plant pathogenic organisms that affect seed germination and seedling stand. Several formulations of metalaxyl-M + fludioxonil seed treatments were tested to investigate their compatibility with the rhizobium seed inoculant.

Inoculant cultures may be applied either directly to the seed, or to the soil, or may be watered onto the soil at or after planting. In light of the limited survivability of rhizobium the most preferred application procedure is to apply the inoculants directly on to the seed before planting. For this reason two application methods viz. a co-applied slurry and a sequential application of various formulations of metalaxyl-M + fludioxonil seed treatment with a commercially available rhizobium inoculant were tested.

MATERIALS AND METHODS

Laboratory Study

Metalaxyl-M + fludioxonil (registered as ApronMaxx™ RTA) was formulated as a ready to use formulation containing metalaxyl-M + fludioxonil 11.45+7.6 g a.i/l. The rhizobium used was a water based formulation of Cell Tech 2000™, applied at the rate of 2.75 ml/kg seed. In addition to metalaxyl-M + fludioxonil the commercial seed treatments used were: Allegiance™ (metalaxyl 320 g a.i./l), Protector L™ (thiram144 g a.i./l), Rival™ (captan + pentachloronitrobenzene (PCNB)+thiabendazole (TBZ) (240+100+10 g a.i./l), Vitavax TL™ carboxin + thiram (340+140 g a.i./l).

The seed treatment formulations were tank-mixed with the rhizobium inoculant culture and applied directly to soya bean seed (variety S20-B9) using a Hege seed treater. Subsequently, the rhizobia on the seed were tested for survivability by plating the seed in YEM bacterial medium at 1, 4 and 24 hours following seed treatment application. The colony forming units (cfu) were counted at 1, 4 and 24 hours after treatment. The data was collected by counting the cfu following incubation at 28°C. The counts were taken when the colonies were clearly visible in the plates.

Field Study

Several variants of the formulation of metalaxyl-M + fludioxonil were included in this study as was co-application with molybdenum as sodium molybdate containing 0.8 g a.i./l of molybdenum. Molybdenum is applied as a seed treatment supplement in high acidic soils and that has become a requirement in soya bean seed treatments in some areas. The formulated seed treatments and the rhizobium inoculant. were applied using a Hege seed treater. The seed treatments were either applied directly as a tank-mix of the rhizobium inoculant with the fungicide seed treatment or sequentially by applying first the seed treatment and then the inoculant.

The treated soya beans were sown in prepared seed beds. The watering and nutrition requirements were followed according to good agronomic practices. Thirty seven days after sowing a subset of soya bean plants were dug and the nodules were counted.

RESULTS

In the laboratory study the survivability of rhizobium inoculant, co-applied as a tank-mix seed treatment slurry with metalaxyl-M + fludioxonil, sustained a higher than expected level of rhizobia per seed compared to the other standard fungicide seed treatments tested (Table 1). The level of survival was comparable to that achieved by rhizobium + water. Twenty four hours after seed treatment the cell counts averaged at 630,000 cell counts per seed from the metalaxyl-M + fludioxonil seed treatment compared to 7000 to 187,000 cell counts from the other seed treatment combinations. It is considered that a rhizobium cell count of 100,000 or over per soya bean seed is optimum.

In the field study the number of the rhizobia root nodules in the soya beans treated with variant 990976 and variant 990978 of the metalaxyl-M + fludioxonil formulation, tank-

mixed with the rhizobium inoculant, was significantly higher than that of untreated and of the original metalaxyl-M + fludioxonil formulation. These variants showed significant improvements over the existing commercially available seed treatment for soybeans. (Table 2). The results study show that the addition of molybdenum (variant 990979)) did however, reduced nodulation significantly compared to variants 990976 and 990978 but not, however, compared to variant 990977.

Table 1. The survivability of rhizobium cells applied to soybean seed with various seed treatment fungicides in a laboratory study.

Treatment	Rate applied g a.i./100 kg seed	Rhizobia/Seed X 1000 colony forming units (cfu)			% difference vs Rhizobium alone		
		1 hr	4 hr	24 hr	1 hr	4 hr	24 hr
Rhizobium		890	660	460	-	-	-
Rhizobium + Water		1,110	890	650	+24.7	+34.8	+41.3
Rhizobium + Metalaxyl-M + Fludioxonil *	3.75 + 2.5	1,190	900	630	+33.7	+36.4	+37.0
Rhizobium + Carboxin + Thiram	51+ 21	290	159	17	-67.4	-75.9	-96.3
Rhizobium + Carboxin + Thiram + Metalaxyl	51+ 21+ 4	400	204	83	-55.1	-69.1	-82.0
Rhizobium + Thiram	70	200	81	19	-77.5	-87.7	-95.9
Rhizobium + Thiram + Metalaxyl	70+ 4	190	94	7	-78.7	-85.8	-98.5
Rhizobium + Captan + PCNB+ TBZ	62+ 26+ 3	360	160	59	-59.6	-75.8	-87.2

*combined formulation of metalaxyl-M + fludioxonil
Figures represent arithmetic means.

Table 2. Effect of metalaxyl-M + fludioxonil (3.75+2.5 g a.i./100kg) seed treatment formulation variants, when applied with the rhizobium inoculant, on the formation of root nodules on soya bean, in a field study.

Treatment	Formln. variant number	Application	Nodules /plant. 37 days after planting
Untreated			3.4
Rhizobium			12.5
Rhiz + meta/flu[A]		Sequential	3.0
Rhiz + meta + flu[B]		Sequential	9.0
Rhiz/meta/flu[C]	990977	Tank-mix	8.9
Rhiz/meta/flu[C]	990976	Tank-mix	11.4
Rhiz/meta/flu[C]	990978	Tank-mix	12.2
Rhiz/meta/flu[C]+molybdenum	990979	Tank-mix	7.9
LSD (0.05)			3.4

[A] = Combined formulation of metalaxyl-M + fludioxonil
[B] = Tank-mix formulation of metalaxyl-M + fludioxonil
[C] = Combined formulation of metalaxyl-M + fludioxonil + Rhizobium

DISCUSSION

The laboratory study demonstrates the marked improvement of rhizobia survivability, 24 hours after soya bean seed treatment, when the inoculant was tank-mixed with metalaxyl-M + fludioxonil compared to the rhizobium treatment alone. This improvement was comparable to that achieved when the inoculant was applied with additional water (which probably reduced water loss from the rhizobia on the seed) and was far superior to the substantial losses which occurred when the rhizobium was applied in tank-mix combination with other commercial fungicide seed treatments.

The field study demonstrates that variants of metalaxyl-M + fludioxonil formulation, when tank mixed with the rhizobium inoculant, can achieve even higher rates of nodulation. A combination of molybdenum with metalaxyl-M + fludioxonil was however, less effective.

This is the first report of the high level of compatibility of soya bean fungicide seed treatments with seed applied rhizobia inoculants. Additional studies are required to determine the actual benefits of such compatibility in field trials.

Effectiveness of carboxin + thiram against seed-borne *Fusarium* spp. in bread and durum wheat

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ABSTRACT

Seed health tests performed on six samples of bread and durum wheat revealed the presence of *Microdochium nivale* and a complex of *Fusarium* species including important pathogens of cereals such as *F.avenaceum*, *F.culmorum*, *F.graminearum* and *F.poae*. Seeds treated with either carboxin + thiram 60 + 60 g a.i./100kg or fludioxonil 5 g a.i./100kg of seed or untreated seeds were incubated in test tubes containing 10 ml water agar, at four different temperature cycles, then examined for the presence of symptoms. Both fungicides were able to control seed-borne *Fusarium* spp. giving rise to a higher number of healthy seedlings. However, only carboxin + thiram reduced the number of asymptomatic plants harbouring endophytic *Fusarium spp*. Field experiments in Central Italy (Viterbo) showed that the two fungicides provided good control of seed-borne pathogens and significantly enhanced emergence and yield in both durum and bread wheat.

INTRODUCTION

There are five main species of *Fusarium* pathogenic to wheat and other cereal crops: *Fusarium avenaceum*, *F.culmorum*, *F.graminearum*, *F.poae* and *M.nivale* (syn. *Fusarium nivale*). These species commonly cause seedling blight, *Fusarium* foot rot and *Fusarium* ear blight. Symptoms on plants may result from infection by either a single species or two or more species (Clement & Parry, 1998). Infection, which arises from soil- as well as from seed-borne inoculum, may cause reduction of seedling emergence and final yield (Cristani, 1992; Hare *et al.*, 1999). Carboxin + thiram is a seed treatment fungicide which confers strong protection against several seed- and soil-borne pathogens of winter and spring cereal crops; previous field trials have shown that plants had better growth and disease tolerance (Balmas *et al.*, 1994).

MATERIALS AND METHODS

Assessment of seed infection

Three seed samples of bread wheat (*Triticum aestivum*) cv. Centauro (E24, E25, E33) and durum wheat (*Triticum durum*) cv. Grazia (U2, U3, U4), harvested in Italy in 1999, were examined with the aim of detecting pathogenic *Fusarium* spp. 4x100 seeds, either untreated or treated (NaClO 1% active chlorine, 10 minutes), were incubated for seven days at 15°C and 24°C under NUV 12hr/12hr on Nash and Snyder medium (NS) (Tuite, 1969), 10 seeds per dish. Colonies developed on NS were subcultured on PDA and incubated at 24°C, NUV 12 hr/12 hr, for species identification.

Seed treatments

Seeds were treated with either Vitavax Flo (carboxin + thiram FS formulation containing 200 + 200 g a.i./l) at 300 ml/100 kg seed or Celest (fludioxonil FS formulation containing 25 g a.i. /l) at 200 ml/100 kg seed or left untreated. The effects of treatments were evaluated both *in vitro* and in field experiments.

***In vitro* assessments of seed treatment efficacy**

Efficacy of treatments was assessed by the seedling symptoms test (Khare *et al.*, 1977). A factorial design with three replicates in randomised blocks was applied. Three replicates of 40 seeds, per each treatment x wheat species x temperature combination, were placed on the surface of 10 ml, 2% water agar in 160 x 16 mm test tubes, one seed per tube, and incubated for 21 days at four different 12 hours cycles of temperature with white fluorescent illumination. Cycles of temperatures were 10-18°C, 13-21°C, 16-24°C, 19-27°C. At the end of the experimental period the number of non-germinated seeds and of symptomatic seedlings (showing streaks or browning of the stem-base) was recorded, germination and healthy stand percentages were accordingly calculated. Percentages were subjected to ANOVA after angular transformation. To check the presence of *Fusarium* spp. within asymptomatic plants, healthy plants from 10-18°C and 19-27°C experiments were then aseptically drawn from the substrate, cut at the crown, and a 2 cm portion of the stem base was pre-treated (1% NaOCl, 30 seconds), rinsed in sterile distilled water and plated on NS medium. Plates were incubated for 7 days at 24°C and NUV (12/12 hours) illumination. Identification was made after PDA subculturing.

Field trial

The field trial was conducted in the 1999-2000 growing season at the experimental farm of the University of Tuscia at Viterbo. A factorial design with three replicates in randomised blocks was applied, the factors being: a) two wheat species, b) three seed treatments.
After autumn ploughing the soil was given 138 kg/ha P_2O_5, 54 kg/ha N and harrowed prior to sowing. Wheat was sown in rows 15 cm apart (628 seeds/m^2), using a plot drill on 15 December 1999. Nitrogen was top dressed once at the rate of 50 kg/ha. A sample area of 1 m^2 was taken for phytopathological and yield measurements. The crop was harvested when the grain moisture content was approximately 10%. The crop was not irrigated.
The phytopathological analysis was carried out at emergence and at tillering on ten plants per plot. Fungal and oomycete pathogens were isolated from sterilised crown tissues on potato dextrose agar (PDA). Carnation leaf agar (CLA) was used to induce sporulation of most *Fusarium* spp. The effects of the seed treatment on plant number, yield, hectolitre weight and 1000-grain weight were determined and subjected to analysis of variance.

RESULTS

***In vitro* assessment of seed infection**

The seed health test, by using NS medium, allows the isolation of many *Fusarium* species and of *M. nivale* from samples of wheat. Among *Fusarium* species the presence of important pathogens of cereals such as *F.avenaceum*, *F.culmorum*, *F.graminearum* and *F.poae* was noteworthy. The occurrence of the isolated *Fusarium* was variable and found to be very high in some samples, e.g. 76.8% of *F.graminearum* in sample U2 and 52% of *F.poae* in sample

E25. Several other *Fusarium* species with no remarkable pathogenic interest for wheat were found. Among them a group of species belonging to section *Liseola* was always present, sometimes at high levels. All samples showed the presence of *M.nivale* but infection levels were not very high, ranging from 0.5% to 22.5% (Table 1).
Incubation temperatures and seed treatment greatly affected species detection. *Fusarium* species as a whole showed higher seed infection percentages when untreated seeds were incubated at 24°C. *M .nivale* detection was positively affected either by seed treatment or by incubation at 15°C. These last results reflect a high sensitivity of *M. nivale* to the presence of other species and its deeper location in seed tissues.

Table 1. Species detection and maximal seed infection percentages of 3 samples of durum and bread wheat.

	Durum wheat			Bread wheat		
Species	U2	U3	U4	E24	E25	E33
F. avenaceum	10.5	12.5	1.3	11.3	10.5	27.5
F. culmorum	1.3	3.5	2.0	0.3	1.3	1.0
F. graminearum	76.8	18.0	1.3	7.8	5.5	2.3
F. poae	5.5	8.5	35.0	10.0	52.0	41.8
F. tricinctum	0.0	1.3	2.0	0.0	0.3	1.0
F. sporotrichioides	0.5	0.5	0.0	2.8	0.5	0.0
F. chlamidosporum	0.5	7.0	0.8	1.3	0.0	0.0
M. nivale	16.5	17.5	8.0	22.5	0.5	6.0
F. equiseti	4.0	9.0	1.3	5.8	5.0	6.5
F. semitectum	0.0	0.8	0.0	6.0	0.0	0.0
"Liseola"	25.3	49.5	22.5	13.0	4.0	3.5

In vitro assessment of seed germination

Among the three main sources of variability, both temperature of incubation and wheat species significantly affected the germination of tested samples, whilst treatments did not influence this parameter. Neither first nor second order interactions were significant. In table 2 averaged data for main sources of variation, ANOVA and Tukey test results are summarised.

Table 2. Germination percentages of wheat samples. Data have been pooled according to the main sources of variation. In each column figures followed by the same letter are not significantly different for $P \leq 0.05$.

Wheat species	%	Temperature	%	Treatment	%
U3	85.9 a	19-27°C	87.7 a	Untreated	90.9 a
U2	87.4 a	13-21°C	91.9 ab	Fludioxonil	91.0 a
U4	89.2 a	10-18°C	93.9 b	Carboxin+thiram	92.6 a
E33	92.5 b	16-24°C	92.6 b		
E24	96.6 bc				
E25	97.6 c				

In vitro assessment of seed treatment efficacy on healthy seedlings

All the three main sources of variability, treatments, temperatures of incubation and wheat species significantly affected the number of healthy seedlings. Neither first nor second order

interactions were significant. In table 3 averaged data for main sources of variation, ANOVA and Tukey test results are summarised.

Table 3. Percentages of healthy seedlings from wheat samples. Data have been pooled according to the main sources of variation. In each column figures followed by the same letter are not significantly different for $P \leq 0.05$.

Wheat species	%	Temperature	%	Treatment	%
U3	78.2 a	19-27°C	82.9 a	Untreated	80.2 a
U2	79.8 a	13-21°C	85.6 ab	Fludioxonil	87.7 b
U4	83.2 a	10-18°C	87.2 b	Carboxin+thiram	90.0 b
E33	89.5 b	16-24°C	88.4 b		
E24	91.6 bc				
E25	93.8 c				

In vitro assessment of asymptomatic seedlings

The presence of pathogens in asymptomatic seedlings from tubes incubated at 10-18°C and 19-27°C, was assessed at the end of the experiment, by plating them on NS medium (Table 4). Several *Fusarium* species and *M.nivale* were isolated, both from plants from both treated and untreated seed. Sometimes *Fusarium* species were isolated from healthy seedlings at high percentages, especially for durum wheat samples. However, *Fusarium* spp. from section *Liseola* frequently represented the majority of detected species. Plants from seeds treated with carboxin + thiram showed a lower percentage of total internal infection.

Table 4. Presence of *Fusarium* spp. in asymptomatic seedlings from the seedling symptom test. Data shown are percentages of infection of plated seedlings. U: untreated; CT: carboxin + thiram; F: fludioxonil.

	Durum wheat						Bread wheat					
	10-18°C			19-27°C			10-18°C			19-27°C		
Fusarium species	U	CT	F	U	CT	F	U	CT	F	U	CT	F
F. avenaceum	3.1	0.7	1.9	4.9	1.0	1.5	13.3	0.6	5.5	8.2	1.8	3.4
F. culmorum	0.0	0.0	0.3	0.0	0.3	0.4	0.0	0.3	0.0	0.7	0.0	0.0
F. graminearum	1.1	1.0	0.0	1.8	0.7	1.1	0.4	0.6	0.0	1.3	0.6	0.0
F. poae	2.4	1.0	1.2	0.4	3.5	2.2	2.5	1.7	4.0	8.4	3.9	10.1
F. tricinctum	1.4	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.6	0.0	0.0	0.0
F. sporotrichioides	0.7	0.0	0.0	0.0	0.0	0.0	4.9	0.0	0.0	0.3	0.0	0.0
M. nivale	2.4	1.0	1.2	0.4	1.0	0.0	2.5	0.6	0.9	2.3	0.0	0.0
F. chlamydosporum	0.7	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
F. equiseti	2.5	0.3	0.6	0.4	0.0	0.0	2.4	0.3	0.9	0.7	0.0	0.6
F. semitectum	0.7	0.0	0.0	0.4	0.0	0.0	0.0	0.3	0.0	0.0	0.3	0.3
"Liseola"	9.3	5.3	35.0	53.9	29.7	75.4	0.7	0.9	10.4	9.0	4.4	17.5
TOTAL	24.2	9.3	40.3	62.5	36.2	80.6	27.1	5.1	22.4	31.9	11.0	31.9
Total excluding *Liseola*	14.9	4.0	5.3	8.6	6.5	5.2	26.5	4.3	12.0	22.9	6.6	14.4

Field trials

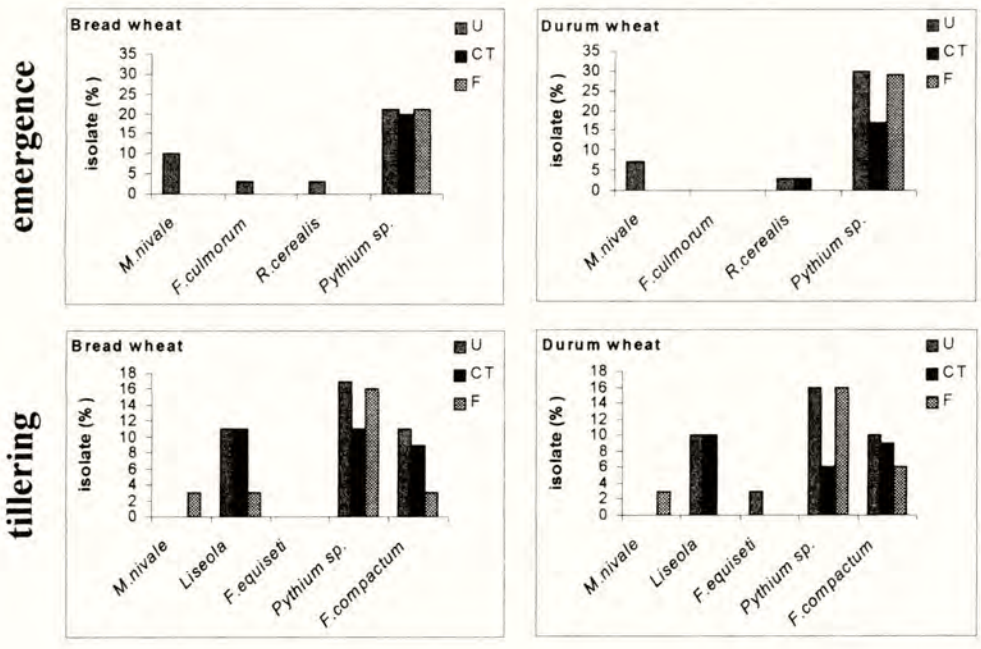
Emergence and yield, for both cultivars, treated with the two products were significantly higher than in the untreated (Table 5). Nevertheless, the other two qualitative indices, hectolitre weight and 1000 grain weight were found to be statistically similar.

Table 5. Effects of seed treatment on production parameters in durum and bread wheat. Means within columns are significantly different at: * $P \leq 0.05$; ** $P \leq 0.01$.

Treatment	Durum wheat				Bread wheat			
	plant/m^2	yield (t/ha)	hectolitre weight (kg)	1000 grain weight (g)	plant/m^2	yield (t/ha)	hectolitre weight (kg)	1000 grain weight (g)
Untreated	257	4,45	79,5	36,40	349	5,18	76,2	34,68
Fludioxonil	367*	5.35**	79,8	35,25	491*	6.32**	76,9	34,61
Carboxin+thiram	374*	5.54**	79,8	36,21	459*	6.46**	76,6	33,30

At emergence (Figure 1), *M.nivale, Rhizoctonia cerealis* and, at higher levels, *Pythium* sp., were recorded from untreated plants of both cultivars; *Fusarium culmorum* was detected in some bread wheat untreated plants. Both durum and bread wheat, but particularly fludioxonil treated durum, were affected by *Pythium* sp.; a low percentage of *R. cerealis* isolates were recorded from durum wheat treated with carboxin + thiram.
At tillering (Figure 1), *Pythium* sp., *F.compactum* and different species belonging to the section *Liseola* were recorded from untreated plants of both cultivars.

Figure 1. Fungal and oomycete soil-borne pathogens isolated at emergence and at tillering from bread and durum wheat.



Durum wheat untreated plants were also affected by a low percentage of *F.equiseti*. Bread wheat treated with both products was affected by the same pathogens recorded from untreated except *M.nivale*, recorded in fludioxonil treated plants. Fludioxonil reduced *F.compactum*. *Pythium* sp. and *F.compactum* were observed in durum wheat treated with both products although carboxin + thiram gave a substantial reduction of *Pythium* sp.. *M.nivale* and *Liseola* isolates were found also from fludioxonil and carboxin + thiram treated plants, respectively.

DISCUSSION

Seed treatment is a very efficient way to control seedling disease such as wheat foot rot. In spite of the complex composition of *Fusarium* population in/on seeds, *in vitro* tests indicated that both carboxin + thiram and fludioxonil, without significant differences between them, were able to control seed-borne *Fusarium* spp. giving rise to an increased healthy stand. Asymptomatic plants from untreated seeds harbour endophytic *Fusarium* spp., of the two chemicals only carboxin + thiram appreciably reduced internal infection.

Field trials gave results that agreed with those obtained by *in vitro* tests. Both fungicides provided good control of seed-borne *Fusarium* pathogens. However, where soil-borne *Pythium* was present carboxin + thiram gave superior control to fludioxonil. Carboxin + thiram not only prevented seed- and soil-borne pathogens but also significantly enhanced emergence and yield in both durum and bread wheat.

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