Session 3
Cereal Seed Treatments

Chairman	R A NOON
Session Organisers	R A NOON J N OAKLEY

CGA 219417: A NOVEL FUNGICIDE FOR THE CONTROL OF *PYRENOPHORA* SPP. ON BARLEY

N.J. LEADBITTER, B. STECK, L.R. FRANK

Ciba Plant Protection, Basle, Switzerland

A.J. LEADBEATER

Ciba Agriculture, Whittlesford, Cambridge, UK

ABSTRACT

CGA 219417 is a novel fungicide of pyrimidine amine chemistry, being developed by Ciba. As a seed treatment CGA 219417 controls leaf stripe (*Pyrenophora graminea*) and seed-borne infections of *Pyrenophora teres* on barley.

Trials in Europe (1990-1993) have shown that CGA 219417 gives excellent control of leaf stripe, equivalent to imazalil, on both winter and spring barley at high and low disease pressure using a rate of 5g AI/100kg seed. Control of seed-borne *P. teres* is also given at 5g AI/100kg seed.

INTRODUCTION

Seed treatment provides an effective and efficient way of applying small quantities of fungicides where they are most needed and is the only efficient method for controlling many seed-borne diseases, for example *Ustilago* spp and *Tilletia* spp.

Leaf stripe of barley caused by *Pyrenophora graminea* S. Ito & Kuribay is an example of a seed-borne disease which can cause great damage in the major barley growing areas of the world (Mathere, 1982). *P. graminea* causes infected plants to be stunted and produces characteristic necrotic striping on most of the leaves which develop. In many infected plants the spikelet does not emerge and where it does little or no grain is produced.

P. graminea survives exclusively as seed-borne mycelium in the hull, pericarp and seed coat. At the time of heading and under conditions of high moisture, conidia are produced on infected leaves and are windblown to nearby heads. Seed may be infected at all stages of development although the early infections are the most severe. Infection of the seedling from the seed is greatly affected by soil temperature and soil moisture. The maximum number of seedlings is infected at temperatures below 12°C (Teviotdale & Hall, 1976) and infection tends to be greatest when the soil is moist rather than wet or dry (Prasad *et al*, 1976).

If left uncontrolled leaf stripe can develop rapidly via infected seed lots and therefore to protect future crops it is important to use a seed treatment to control the disease. Since the 1940s leaf stripe has been successfully controlled using mercury seed treatments, except in the rare cases where resistance of the fungus to mercury developed. Following the banning of mercury throughout Western Europe several products have been used which give incomplete control of the disease. Most recently the phenylpyrroles (fenpiclonil and fludioxonil) have been added to this armoury. For very high levels of control e.g. on seed crops, a single active ingredient, imazalil, has often been relied upon.

Net blotch (*Pyrenophora teres* Drechs.) is a common disease of barley everywhere the crop is grown. The disease is known in two forms, the "net" type, the most common form which gives net-like symptoms on the leaves and the "spot" form which produces dark brown elliptical lesions surrounded by a chlorotic area (Mathere, 1982). Seed-borne infection can be the primary source of inoculum from which lesions develop on the lower leaves and coleoptile. From these lesions conidia are then dispersed to cause further infection on newly developing foliage. Under favourable conditions large areas of the leaf tissue can be affected and hence yield reduced. Infection of the seed occurs when conditions allow the ear to be infected during seed development. Control of the seed-borne infection is mainly by products containing imazalil.

New compounds that will broaden the chemical basis of control of *Pyrenophora* spp. on barley are therefore desirable. This paper describes the activity of a novel compound CGA 219417 when used against *P. graminea* and *P. teres* on barley.

PRODUCT

CGA 219417 (proposed common name cyprodinil) is a new pyrimidine amine fungicide which is being developed by Ciba for the control of leaf stripe on barley as well as for the control of a range of foliar diseases on grapes, cereals and other crops (Heye *et al*, in press). This compound shows good activity against pathogenic fungi in the *Ascomycetes* and *Deuteromycetes*. Biochemical studies show that CGA 219417 inhibits amino-acid synthesis (*Ibid*). As such it has a different mode of action from other products which control leaf stripe on barley.

METHODOLOGY

Replicated field trials were carried out in a number of European countries from 1990 to 1993 using naturally infected seed. The levels of infection on the seed were determined before treatment.

Treatments were usually applied diluted in a Hege 11 seed dresser. Seed loading analyses were made on selected batches following treatment to evaluate the quantity of CGA 219417 on the seed.

CGA 219417 formulated as either an FS025 or ES025 water based formulation containing 25g AI/litre was applied at a product rate of 0.2litre/100kg of seed. The following standards were used. On winter barley, triadimenol+fuberidazole+imazalil (37.5+4.5+5g AI/100kg seed; GB, 1990 & 1991, CH & D, 1991-1993), triadimenol+imazalil (11.3+4.5g AI/100kg seed; I, 1990 & 1991), fenfuram+guazatine+imazalil (30+60+4g AI/100kg; D, 1990, CH, 1991), fenpiclonil+imazalil (20+4g AI/100kg seed; GB, 1992), flutriafol+ethirimol+thiabendazole+imazalil (15+200+5+4g AI/100kg seed; GB, 1993) and betaxate+anthraquinone (20+50g AI/100kg seed, F, 1991 & 1992). On spring barley, triadimenol+fuberidazole+imazalil (37.5+4.5+5g AI/100kg seed; D, 1992); fenpiclonil+imazalil (20+4g AI/100kg seed; D, 1991, CH, 1990); triadimenol+fuberidazole (37.5+4.5 AI/100kg seed; GB, 1991); guazatine+imazalil (60+5 g AI/100kg; GB, 1993).

Per cent crop vigour was assessed using a visual score for each plot. This evaluation gives an overall evaluation of plant size, crop cover and crop appearance in comparison with other plots in the trial. In Germany the untreated plots were always given a 100 rating and other plots in the replicate assessed in comparison with these. In this case treated plots can have values greater than 100 if they are more vigorous than the untreated. In Great Britain the most vigorous plot in the replicate was assessed as 100 and other plots in the replicate assessed in comparison with it. In this case treatments can have values greater than the untreated if they are more vigorous but the value will never exceed 100. Assessments of plant stand were made by counting the number of plants in five randomly chosen 0.5m lengths of row in each plot. Leaf stripe was assessed by counting the number of infected tillers at ear emergence. Infection by *P. teres* from seed-borne infection was made at Zadoks GS10 - 20 by counting numbers of diseased plants.

RESULTS AND DISCUSSION

Crop tolerance

Field trials carried out during 1990 - 1993 in Germany, Italy, France, Switzerland and Great Britain showed that CGA219417 when used at 5g AI/100kg seed caused no adverse effects on plant stand, subsequent plant development or final yield (results not presented). Data for crop development (Table 1) also showed that when CGA 219417 was used at 5 and 7.5g AI/100kg seed there were no adverse effects on plant development compared with the untreated under conditions of rapid growth (Germany, Sept/Oct drilling) or where crop development was slower due to later drilling (UK, Oct/Nov drilling). In some cases, under the same conditions crop vigour had been reduced by the standard.

TABLE 1. Crop tolerance of CGA 219417 on winter barley, measured as % crop vigour* at Zadoks growth stage 10-11.

Treatment	Rate g AI/100kg seed	Country D	D	GB	GB
untreated	-	100	100	85.7	90.7
standard	*	90	100	38.8	36.3
CGA 219417	5.0	100	100	91.3	86.3
CGA 219417	7.5	100	100	91.3	86.9
Days after planting		13-26	24-26	22-35	55
Drilling date		26/9-19/10	21/9-28/9	24/10-31/10	15/11-28/11
No. of trials		5	4	2	2
Years		90	91	90	91

* see methodology

In 1993, trials using certified seed were conducted in Great Britain to assess the crop tolerance of CGA 219417 at 10g AI/100kg on eight varieties of winter barley. The data show that CGA 219417 at this rate does not adversely effect plant stand (Table 2)

TABLE 2. Crop tolerance of CGA 219417 on 8 winter barley varieties in Great Britain, 1993. Numbers of plants per 0.5m length of row.

Treatment	Rate g AI/100kg	Variety** 1	2	3	4	5	6	7	8
untreated	-	30.9	34.6	37.2	30.1	30.8	29.8	27.3	31.0
standard*	220	26.2	31.8	35.2	26.3	27.7	25.1	23.8	28.2
CGA219417	10	28.8	33.6	36.4	28.0	34.4	27.1	28.2	31.8
LSD(p=0.05)***		5.64	5.41	5.39	7.98	6.59	5.46	6.38	7.87

* flutriafol+ethirimol+thiabendazole 15+200+5g AI/100kg seed

** 1: Marinka, 2: Puffin, 3: Gypsy 4: Silk, 5: Pipkin 6: Sprite, 7: Princess, 8: Halcyon

*** Comparisons only possible within columns. LSD calculated using Tukey Test.

Activity of CGA 219417 against leaf stripe on winter barley

Field trials carried out in a number of European countries showed that control of leaf stripe on winter barley by CGA 219417 (5g AI/100kg seed) was at least equal to the standard products (Table 3). In all countries except France the standards contained imazalil (4-5g AI/100kg seed depending on the product) demonstrating that the control of leaf stripe given by CGA 219417 was at least as good as the most common material used for control of this disease.

TABLE 3: % Control of *P. graminea* on winter barley in European countries using CGA 219417.

Treatment	Country CH	D	GB	F	I
untreated	(16.1)*	(7.7)*	(8.7)*	(37.8)*	(8.4)*
standard **	98.8	97.3	98.8	75.8	91.6
CGA 219417 ***	98.5	98.2	99.3	96.9	96.7
No. of trials	11	16	8	5	6
Years	90-92	90-92	90-93	91-92	90-91

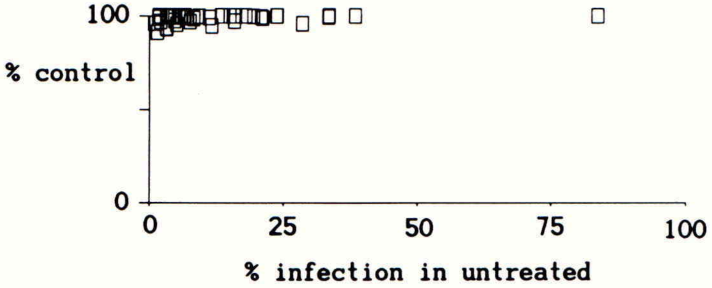
* % infection in untreated

** see Methodology

*** Applied at 5g AI/100kg seed

In these trials a wide range of infection levels occurred, demonstrating that at 5g AI/100kg seed CGA 219417 gives consistently high levels of disease control independent of disease pressure (Figure 1).

FIGURE 1. % Control of *P. graminea* on winter barley by CGA 219417 (5g AI/100kg seed) as related to final disease levels in the untreated (data from 41 trials).



Activity of CGA 219417 against leaf stripe on spring barley

In field trials in Switzerland, Germany and Great Britain CGA 219417 (5g AI/100kg seed) also gave excellent activity against leaf stripe on spring barley, equivalent to the standards (Table 4).

TABLE 4. % Control of *P. graminea* on spring barley in European countries using CGA 219417

	Country		
Treatment	CH	D	GB
untreated	(10.1)*	(12.1)*	(10.8)*
standard **	100	99.8	100
CGA 219417 **	99.6	99.5	99.6
No. of trials	1	3	3
Years	90	91-92	91 & 93

* % infection in untreated
** see Methodology
*** Applied at 5g AI/100kg seed

Activity of CGA219417 against *P. teres* on barley

In trials carried out in Denmark in 1991 and 1992 CGA 219417 gave very good control of *P. teres* generally equivalent to the standard imazalil (Table 5).

TABLE 5. % Control of *Pyrenophora teres* on spring barley in Denmark

Treatment	Year 1991	 1992
untreated	(8.2)*	(12.7)*
imazalil **	79.3	92.1
CGA 219417 **	71.9	86.6
No. of trials	3	3

* No. of diseased plants per m^2

** Applied at 5g AI/100kg seed

CONCLUSION

Field trials carried out over a number of years and in various countries have demonstrated the excellent and consistent activity of CGA 219417 against leaf stripe of barley. Unlike some other classes of compounds e.g. the triazoles there is no difference in the level of control on winter and spring barley. A high level of control has also been demonstrated against seed-borne infection of *P. teres*. This activity together with its excellent crop safety and low use rate make CGA 219417 a good alternative to imazalil where consistently high levels of leaf stripe control are required, thereby reducing the dependence on a limited number of active ingredients.

With both economic and environmental pressures against the use of agrochemicals continuing to increase seed treatment remains an efficient and environmentally attractive method of disease control. CGA 219417 provides another tool with which to develop future crop protection strategies.

ACKNOWLEDGEMENTS

The authors would like to thank their colleagues from Italy, Great Britain, Germany and France who carried out the field work described in this paper.

REFERENCES

Heye, U.; Speich, J.; Siegle, H; Wohlhauser, R.; Zeun, R. (1994) CGA 219417: A new broad spectrum pyrimidine amine fungicide for cereals, pome fruits, grapes and other crops. *Crop Protection* In press

Mathre, D.E. (1982) Barley stripe, In: *Compendium of barley diseases*, Mathre, D.E. (Ed), American Phytopathological Society, Minnesota.

Prasad, M.N.; Leonard, K.J.; Murphy, C.F. (1976) Effects of temperature and soil water potential on expression of barley leaf stripe incited by *Helminthosporium gramineum*. *Phytopathology*, 66, 631-634.

Teviotdale, B.L.; Hall, D.H. (1976) Factors affecting inoculum development and seed transmission of *Helminthosporium gramineum*. *Phytopathology*, 66, 295-301

EFFICACY OF TRITICONAZOLE SEED TREATMENT AGAINST COMMON EYE-SPOT IN WHEAT.

J.M. GAULLIARD

Rhône-Poulenc Agro France, 55 avenue René Cassin, CP 310, 69337 Lyon
Cedex 09

M. CHAZALET, A. SAILLAND, J.M. GOUOT

Rhône-Poulenc Agro, 14/20 rue Pierre Baizet, BP 9163, 69263 Lyon Cedex 09

ABSTRACT

Triticonazole (RPA 400727) is a new triazole fungicide developed by Rhône-Poulenc Agro for seed treatment of cereals and other crops. Because of its excellent selectivity, seeds treated at 1200 g AI/t were protected against early attacks of cereal diseases and the programme of subsequent foliar fungicide applications could therefore be reduced. As observed in a number of trials conducted in France, triticonazole was effective against common eye-spot (*Pseudocercosporella herpotrichoides*), from emergence and during the most damaging period of the cropping season. It controlled early attacks of W (fast-growing) and R (slow-growing) strains, decreasing both the incidence and the severity of the disease.

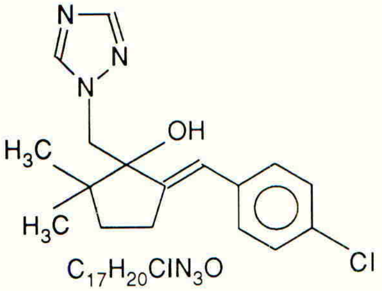
INTRODUCTION

Rhone Poulenc Agro have discovered a new sterol demethylation inhibitor (DMI) fungicide which has shown high selectivity in cereals, especially by seed treatment, superior to most other DMI products. Some of the properties of triticonazole (RPA 400727) have been described already (Mugnier *et al.*, 1991,1993). The use of a high dose on the seeds allowed better control and a longer protection of cereal diseases such as common eye-spot caused by *Pseudocercosporella herpotrichoides* (Montfort et *al*, 1991). Work to confirm this is reported in this paper.

Physical and chemical properties of triticonazole are described below :

Chemical name (IUPAC)	(1RS)-(E)-5-(4-Chlorophenyl)methylene)-2,2-dimethyl-1-(1H-1,2,4-triazol-1-ylmethyl)-cyclopentan-1-ol

Chemical structure



$C_{17}H_{20}ClN_3O$

Molecular mass:	317.82
Melting point:	144°C
Vapour pressure:	< 0.1 x 10-7 hPa (50°C)
Solubility in water:	8.4 mg/l (20°C)
Partition coefficient: (Octanol/water)	logP = 3.29

Toxicology

Acute and subchronic toxicological studies have demonstrated that triticonazole shows a very low toxicity towards mammals (Table 1).

TABLE 1. Acute toxicity of triticonazole.

Animal	Route	Results
Rat (M + F)	Oral	LD 50 : > 2000 mg/kg
	Dermal	LD 50 : > 2000 mg/kg
	Inhalation	LC 50 : > 1.4 mg/kg
Rabbit	Eye	Irritant
	Skin	Non irritant
Guinea pig	Skin	No sensitization

Ecochemistry

In standard laboratory conditions, there was no significant hydrolysis of triticonazole in water (pH range : 5-9). The product could be unstable under strong light in water solution or in some organic solvents in the presence of chemical photoactivators but it was stable when applied on seeds stored under standard conditions.

In standard laboratory conditions, its half-life in a clay loam soil was of the order of 4 months. Triticonazole was neither toxic to birds (LD 50 > 2000 mg/kg) nor to the total microbial soil population.

Formulations

Several formulations of triticonazole have been developed. These are mainly suspension concentrates (FS), alone or in mixture with complementary fungicides such as guazatine, iprodione, or with anthraquinone as a bird repellent for specific countries and uses. In France, an FS containing 200 g triticonazole + 84 g anthraquinone / litre has received a provisional registration in 1993 and is commercialized under the trade mark REAL. The registered dose rate for this formulation is 6 l/t of seeds, for the control of the following seed-borne diseases : *Tilletia caries*, *Septoria nodorum*, *Fusarium roseum* on wheat and *Ustilago nuda* on barley . The following foliar diseases are also controlled : *Erysiphe graminis*, *Septoria tritici* and *S. nodorum*, *Puccinia striiformis* and *P. recondita* on wheat, *Rhynchosporium secalis*, *E. graminis* and *P. hordei* on barley.

Material and methods

Field trials have been conducted in France according to the official French methodology : CEB (Commission des Essais Biologiques) no. 64 with 4 blocks, and 20-50 m^2/plot. Wheat seeds treated with triticonazole at 1200 g AI/t were sown at 160-200 kg/ha. In some trials, the inoculum was increased by broadcasting *P. herpotrichoides*-inoculated straw or grain in the field during winter. The types of strains found on diseased stems were determined by molecular biology techniques. This method allowed the specific characterization of *P. herpotrichoides* from a mixture of other fungi, even in case of very early attacks without symptoms and permitted the differentiation between the W (Wheat type, fast-growing strain) and R (Rye type, slow-growing strains) types of common eye-spot strains. It was then easier to determine the frequency of attacks caused by each strain type in treated and untreated plots. An average of 20 to 40 tillers or stems per plot were assessed.

RESULTS

Characteristics of *P. herpotrichoides* strains in France (1992 and 1993)

Analyses of the strains of the population at many locations (38 locations in 1993) were conducted and used to map their distribution in France according to the W/R type ratio. The R type strains were predominant over W strains in the North and North-West of France, while W strains were in the majority in the rest of the country. R strains were present in most of the triticonazole trials.

Efficacy of triticonazole against common eye-spot

The frequency of both strain types on wheat tillers was determined at growth stage Z 30, and triticonazole efficacy against early attacks of common eye-spot was calculated at this stage using the Abbott formula. As shown in Table 2, triticonazole was effective against both W and R strains which were found alone or mixed on leaf sheaths, 30 out of 38 locations showing at least 5% of tillers infected either by a W or an R strain (or by both strains together).

TABLE 2. Efficacy of triticonazole seed treatment (1200 g AI/t) against *P. herpotrichoides* on wheat at GS Z 30, as determined by molecular biology techniques (average of 30 locations in France, showing at least 5% infected stems in untreated 1992/93 trials).

	All strains	W type	R type
Efficacy (%)	52.2	58.8	47.5
% infected stems in untreated	32.9	20.9	18.7
Number of trials	30	26	25

TABLE 3. Results from trials conducted in 1992 by R.P. Agro France.

	% of stem section girdled and yield (t/ha) Trial reference code						Means :
Product/dose g A.I/t or ha	trial 1	trial 2	trial n 3	trial 4	trial 5	trial 6	% control yield benefit over untreated t/ha
triticonazole	19.5 b	21.0 b	18.6 b	7.3 bc	25.8 bc	40.9 bc	43.2
1200 g/t	-	(8.72 a)	(5.51 ab)	(6.12 a)	(6.70 ab)	(8.62a)	(0.197)
prochloraz	20.9 b	7.8 c	15.9 bc	13.2 ab	9.6 cd	38.3c	51.3
600 g/ha	-	(8.77 a)	(5.48 ab)	(5.77 ab)	(7.08 a)	(8.00 b)	(0.084)
flusilazole	19.7 b	15.6 bc	15.4 bc	10.6 abc	3.2 d	42.7 abc	50.8
300 g/ha	-	(8.84 a)	(5.33 ab)	(6.20 a)	(7.00 a)	(8.47 ab)	(0.239)
untreated	33.0 a	33.0 a	31.0 a	20.0 a	56.0 a	54.3 ab	
	-	(8.77 a)	(5.192 b)	(5.582 b)	(6.93 ab)	(8.20 ab)	
Inoculation	artificial	natural	artificial	natural	natural	artificial	
LSD %	6.25	9.37	7.95	8.88	18.3	12.59	
CV %	19.34	37.67	33.34	55.84	65.8	19.44	

Figures followed by the same letter are not statistically significantly different (P = 0.05)

Trials conducted by R.P. Agro in 1992 have demonstrated that triticonazole seed treatment at 1200 g AI/t gave a good control of eye-spot, in the range of efficacy achieved by a foliar application of standard EBIs registered against eye-spot in France, bringing an additional yield of 0.2 t/ha on average over the untreated control (Table 3). These results have been confirmed by trials conducted in 1992 by I.T.C.F. - Institut Français des Céréales et des Fourrages - (data not shown). In 1993, a large number of trials have been conducted with triticonazole at 1200 g AI/t. Early assessments have demonstrated the good efficacy of triticonazole against early attacks of eye-spot which occurred very early, from November 1992 and all through the winter and spring seasons (Table 4).

TABLE 4. Efficacy of triticonazole in 1993 R.P. Agro France trials (early assessments, average of 30 trials).

	triticonazole	prochloraz
Application	Seed treatment	Foliar treatment
Dose rate	1200 g AI/ t	600 g AI/ ha
% Efficacy at GS Z30*	44.2	Not applicable
GS Z40*	43.9	42.3
GS Z55-60**	53.0	47.7

* based on frequency of attacked stems (incidence)

** based on severity of the attack (% stem circumference girdled)

Results from most of the trials are not yet available. However, in a series of trials conducted by the RP Agro Evaluation farms, in which a W strain sensitive to EBIs and benzimidazoles has been used, triticonazole showed an acceptable efficacy versus the standard fungicides (Table 5) when observed late in the season (G.S. Z64 to Z75, 192 to 223 days after sowing). In addition, in two of these trials, lodging occurred, following heavy rainfall. In these two cases, triticonazole prevented lodging as well as the standard EBI fungicides (Table 6).

TABLE 5. Efficacy of triticonazole against *P. herpotrichoides* in 1993 R.P. Agro - Evaluation trials (assessments made at G.S. Z64 - Z75).

Trial	Growth stage (days after sowing)	% stem circumference girdled (% efficacy)			
		Untreated	triticonazole 1200 g AI / t	prochloraz 600 g AI / ha	flusilazole+ carbendazim 300 + 150 g AI / ha
1	Z64	70 a	45 b	27 bc	14.5 c
	(192 das)		(37.5)	(61.4)	(79.2)
2	Z64	19a	5.3 bc	3.8 bc	1.3 c
	(226 das)		(72)	(80)	(93.1)
3	Z75	26.1 a	17.4 b	3.6 c	1.5 c
	(180 das)		(33.3)	(86.2)	(94.2)
4	Z75	17.7 a	1.3 b	1.7 b	1.3 b
	(213 das)		(92.5)	(90.2)	(92.5)
5	Z69	28.9 a	21.4 ab	6.0 b	22.3 ab
			(26.0)	(79.2)	(22.8)

Figures followed by the same letter are not statistically significantly different (P = 0.05)

TABLE 6. Effect of triticonazole and other fungicides on winter wheat crop lodging.

% of crop lodging	Untreated	triticonazole 1200 g AI / t	prochloraz 600 g AI / ha	flusilazole + carbendazim 300+150 g AI /ha
Trial 1 (239 das)	37.5 a	3.8 b	0 b	2 b
Trial 2 (245 das)	10 a	0 b	0 b	0 b

Figures followed by the same letter are not statistically significantly different (P = 0.05)

CONCLUSION

Triticonazole seed treatment at 1200 g AI/t, i.e. of the order of 200 g AI/ha when applied on seed drilled at 0.17 t/ha, provided good protection of cereal crops against early attacks of common eye-spot occurring from autumn up to the spring season, controlling both W and R types of strains. These early attacks generally caused most of the yield, losses. justifying the use of triticonazole as a preventive treatment during this period.

REFERENCES

Montfort, F. ; Denis, S. ; Cavelier, N. (1991) Effect of two triazole fungicide seed treatments on eye-spot of cereals caused by two strains of *Pseudocercosporella herpotrichoides* (Fron) Deighton. *Troisième Conférence Internationale sur les maladies des plantes ANPP. Bordeaux*, **III** , 1065 - 1072.

Mugnier, J. ; Chazalet, M. ; Gatineau, F. (1991) RPA 400727: un nouveau fongicide pour le traitement des semences de céréales. *Troisième Conférence Internationale sur les maladies des plantes ANPP. Bordeaux*, **III** , 941-948.

Mugnier, J. ; Gouot, J.M. ; Hutt, J. ; Greiner, A. ; Chazalet, M. ; Gaulliard, J.M.Ingram, G. (1993) RPA 400727, un nouveau traitement de semences efficace contre les maladies foliaires des céréales. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* (in press).

TRIAZOXIDE - A SEED TREATMENT FUNGICIDE FOR THE CONTROL OF SEED BORNE *PYRENOPHORA* SPECIES

S. DUTZMANN

Bayer AG, PF-F/F, Pflanzenschutzzentrum Monheim, D-51368 Leverkusen

ABSTRACT

Triazoxide is a specific non-systemic fungicide belonging to the chemical group of benzotriazines effective against seed borne *Pyrenophora graminea* and *Pyrenophora teres* on barley.With an application rate of 2 - 4 g AI / 100 kg of seed it is well qualified for completing the spectrum of activity of other fungicidal seed dressing compounds. The experience with triazoxide which is already in use in several European countries, is described and discussed.

INTRODUCTION

Cereal fungicides, both seed dressings and foliar sprays, are not only expected to provide a high level of activity but also to cover a broad spectrum of different diseases at the same time. In most cases it is not a single pathogen but a complex of them which may reduce the quality and quantity of yield. Although much effort has been made in that respect, numerous fungicidal compounds do not fully meet these requirements.

A classical gap in control of seed-borne diseases is the lack of activity of many seed dressing compounds against *Pyrenophora graminea*, the cause of leaf stripe on barley. After prohibition of mercury-containing seed dressings, leaf stripe could have become a serious problem had imazalil not been available. In this paper, triazoxide is presented as a novel benzotriazine based fungicide with a specific activity against seed-borne *Pyrenophora* diseases.

CHEMICAL AND PHYSICAL PROPERTIES

Chemical class:	Benzotriazine
Chemical name:	7-chloro-3-(1H-imidazol-1-yl)-1,2,4-benzotriazine-1-oxide
Common name:	Triazoxide
Structural formula:	

Molecular formula:	$C_{10}H_6ClN_5O$
Molecular mass:	247.7 g / mol
Appearance:	light yellow crystals
Melting point:	182° C
Vapour pressure:	20° C: 1.5×10^{-12} Pa 25° C: 5.2×10^{-12} Pa 120° C: 2.4×10^{-4} Pa
Solubility (g / l at 20° C)	water: 3×10^{-2} n-hexane: < 1 dichloromethane: 50 - 100 2-propanol: 2 - 5 toluene: 20 - 50
Stability (water):	stable at ph 4 - 7
(light):	may be decomposed by light

TOXICOLOGY

Acute oral LD_{50} rats:	100 - 200 mg / kg
Acute dermal LD_{50} rats:	> 5000 mg / kg
Skin and eye irritation:	negative
Inhalation toxicity (4 hour LC_{50} rats):	0.8 - 3.2 mg / l
Teratogenicity:	no teratogenic effect to rats and rabbits
Mutagenicity:	in "in-vivo" and "in-vitro" tests no mutagenic effects

FORMULATIONS

Triazoxide can be formulated as a DS-, WS-, LS- and FS-seed treatment. Combinations with triadimenol, tebuconazole, fuberidazol, anthraquinone and bitertanol have been found to be relatively stable.

BIOLOGICAL PROPERTIES

The first tests in the greenhouse soon revealed that triazoxide is a non-systemic fungicide which is very suitable as a seed treatment. The spectrum of activity is mainly restricted to seed-borne *Pyrenophora* diseases (Table 1). Windborne *Pyrenophora*, e.g. *P. teres* is also influenced by applying a foliar spray, but due to the poor systemicity of the active ingredient, curative effects are too weak. Used as a seed treatment with the recommended rate of 2 - 4 g AI / 100 kg of seed, triazoxide perfectly controls *P. graminea* on spring and winter barley as well as seed-borne net blotch (*P. teres*). By contrast, triazoxide (like imazalil) has little effect on *Pyrenophora avenae*, the cause of stripe disease on oats.

TABLE 1. Spectrum of activity of triazoxide as a seed treatment.

Crop	Rate (g AI/100 kg seed)	Activity		
		v. good	good	poor
Barley	2 - 4	*Pyrenophora graminea*	*Pyrenophora teres* (seed-borne)	
Oats	2 - 4			*Pyrenophora avenae*
Wheat	2 - 4			*Tilletia caries*

If used with the recommended maximum rate of 4 g AI / 100 kg of seed, triazoxide displays good seed tolerance either alone or in combination with other seed dressing compounds.

TABLE 2. Efficacy of triazoxide against *Pyrenophora graminea* on winter barley (30 % disease) and on spring barley (48 % disease); Monheim field trial, 1993.

Crop	Treatment	Rate (g AI / 100 kg seed)	Efficacy (%)
Winter barley (cv. Franka)	Triazoxide (2.5 DS)	5	100
		4	100
		3	100
		2	100
	Imazalil (2.5 DS)	5	96
		4	99
		3	98
		2	96
Spring barley (cv. Asse)	Triazoxide (1.0 DS)	5	99
		4	99
	Imazalil (1.0 DS)	5	97
		4	99

As can be seen from Table 2 the performance of triazoxide is at least comparable to that of the standard compound imazalil. Even with the lowest rate of 2 g AI / 100 kg of seed, complete control of *P. graminea* on barley can be expected.

In the course of many field trials in Germany, it has been shown that leaf stripe on spring barley is slightly easier to control than on winter barley (Table 3): unfavourable growing conditions, e.g. low temperatures and unregular water supply during the phases from germination to end of tillering are known to extend the succeptible period for infection by *P. graminea*. Nevertheless, germination conditions in spring and autumn normally do not differ much from each other, thus the date of sowing alone cannot explain the difference in control. Maybe the long period of overwintering creates better conditions for systemic invasion by the fungus, while the growth of the host is slowed down or stopped. Nevertheless, triazoxide still exhibits consistently good control which can also be seen from the little variation in efficacy (figures in round brackets, Table 3).

TABLE 3. Difference in control of *Pyrenophora graminea* on spring and on winter barley.

Crop	Treatment	Rate (g AI/100 kg seed)	Control (%)	Disease (%)	Number of trials
Spring barley	triazoxide + fuberidazol	4 + 4	99.5 (98-100)*	32 (11-68)*	7
Winter barley	triazoxide + fuberidazol	4 + 4	98.8 (91-100)*	9 (1-52)*	19

* = Range

Unfavourable growing conditions may require an application rate of 4 g AI triazoxide / 100 kg of seed, e.g. for late-sown winter barley in Northern and Central Europe. However, this amount of AI may be reduced to 2 g, if the climatic conditions are more favourable (for instance in France). The same can be done, if triazoxide is combined with a seed dressing compound which has already - like tebuconazole - some activity against *P. graminea*.

In contrast to a monocyclic disease like leaf stripe on barley which can be fully eliminated by a single seed treatment, the polycyclic net blotch of barley (*P. teres*) cannot because it is the wind-borne inoculum released from stubble, which determines the severity of an epidemic. However, control of seed-borne *P. teres* is desirable since it may delay the onset of disease.

As can be seen from Table 4, both triazoxide and imazalil are able to control seed-borne *P.teres*. In combination with triadimenol an effect on early wind-borne infection may also be expected due to the high systemicity of triadimenol.

TABLE 4. Control of seed borne *Pyrenophora teres* (glasshouse test, average of 11 cultivars).

	Rate (g AI / 100 kg seed)	Control (%)
Triazoxide	5.0	95.8
	7.5	98.3
Imazalil	5.0	94.3
	7.5	95.6
Triadimenol +	22.5 +	99.1
Triazoxide	7.5	
Triadimenol +	22.5 +	95.2
Imazalil	7.5	
(Untreated mean disease level, %)		(12.1)

CONCLUSION

Triazoxide is a non-systemic fungicide for use as a cereal seed treatment. The above data demonstrate the high level of activity against *P. graminea* and seed-borne *P. teres*. As the dose required is extremely low, triazoxide is well suited for those seed dressings which exhibit a deficiency in control of barley leaf stripe. Combinations with other fungicidal seed dressings are already introduced into the German, French, Belgian, Norwegian and Spanish markets and they are under development for further European countries. Triazoxide brings new chemistry to the small group of compounds like imazalil which control *P. graminea.*

SEED TREATMENTS ON WHEAT IN THE USA USING DIFENOCONAZOLE

A. B. BASSI, Jr., D. LAIRD, L. ZANG

Ciba-Geigy Corporation, Plant Protection Division, P.O. Box 18300, Greensboro, NC, USA

N.J. LEADBITTER

Ciba Plant Protection, Basle, Switzerland

ABSTRACT

Difenoconazole is a triazole fungicide that offers broad spectrum control of seed and foliar pathogens of cereals in the USA when used as a seed treatment. In the USA it is being developed at rates of 6 to 24g AI/100kg seed.

In field trials on wheat difenoconazole has provided excellent activity against loose smut (*Ustilago tritici*), common bunt (*Tilletia caries*), dwarf bunt (*Tilletia contraversa*) and *Septoria* seedling blight (*Septoria nodorum*). In addition difenoconazole will give early season control of leaf rust (*Puccinia recondita*).

Difenoconazole also shows excellent crop safety and together with its good efficacy against a wide range of seed-borne diseases on wheat will provide a new opportunity for disease control using seed treatment in the USA.

INTRODUCTION

Seed-borne and soil-borne pathogens of wheat have become increasingly important in the USA with the advent of no-till farming and wheat mono-culture practices. The Certified Seed Program in the USA provides good quality seed to the grower but is not a 'stand alone' solution. Seed treatments are required to provide control of seed-borne diseases such as loose smut (*Ustilago tritici*), common bunt (*Tilletia caries*) and *Septoria* seedling blight (*Septoria nodorum*). Currently used products are mainly based on carboxin, triadimenol, thiram and carbendazim.

Dwarf bunt (*Tilletia contraversa*) is also a serious problem in parts of the USA i.e. Pacific North West. Due to the soilborne nature of this disease it cannot be controlled by certified seed programs. Until their ban, mercury seed treatments were used for the control of this disease. Currently there are no products registered in the USA for control of dwarf bunt.

Difenoconazole is a systemic fungicide that is highly effective against Ascomycetes, Basidiomycetes and Deuteromycetes (Ruess *et al*, 1989, Dahmen *et al*, 1992). It is currently used as a foliar fungicide in other parts of the world and will be available as a seed treatment in a number of countries. Difenoconazole is a triazole and acts on the C14 demethylation step in ergosterol synthesis.

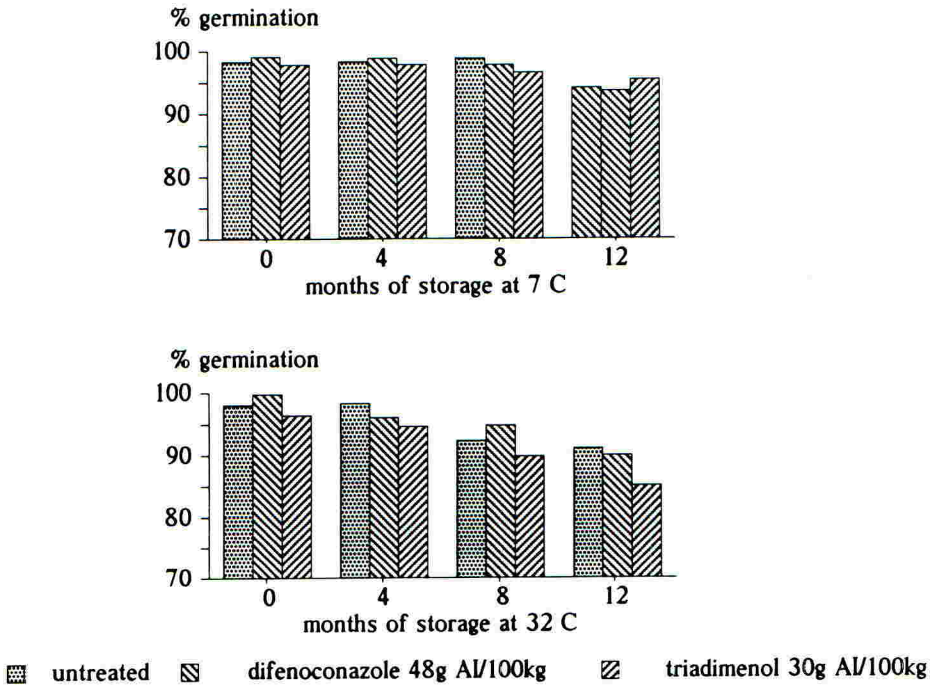
In the USA difenoconazole has shown activity against a wide range of seed-borne diseases including dwarf bunt. Under the trade name Dividend™ it has been submitted to the U.S. Environmental Protection Agency for full registration in the USA as a seed treatment on wheat and barley. This paper gives a summary of its activity on wheat.

CROP SAFETY

Difenoconazole has been tested in field research trials for seven years in the USA. Emergence and crop vigour ratings have been evaluated to check crop safety across a wide range of environmental conditions. Difenoconazole (up to 48g AI/100kg seed) has proven to be non-phytotoxic and does not show any growth regulatory effects (data not shown).

Following treatment using the the commercial formulation of difenoconazole (3FS, 3lbs AI/gallon, 360g AI/litre) at an application rate of 48 g AI/100kg seed, seed was stored for 0, 4, 8 and 12 months at 7 and 32°C. Crop safety was then evaluated by assessing germination after incubation in soil at 18-20°C (Figure 1). The test showed that even after storage under adverse conditions difenoconazole at double the expected maximum application rate is safe to the seed and is generally at least equal to triadimenol (30g AI/100kg) at its standard use rate.

FIGURE 1. The influence on % germination of storage at 7 and 32 °C when wheat seed was treated with difenoconazole.



CONTROL OF DISEASES

Difenoconazole is effective against a range of diseases at rates of 6 - 24g AI/100kg depending on the disease (Table 1).

TABLE 1. Diseases of wheat controlled by difenoconazole when used as a seed treatment in the USA.

Disease	Pathogen	Rate (g AI/100kg seed)
Common bunt	*Tilletia caries*	6
Dwarf bunt	*Tilletia contraversa*	12
Loose smut	*Ustilago tritici*	12-24
Flag smut*	*Urocystis agropyri*	12-24
Septoria* seedling blight	*Septoria nodorum (seed)*	12-24
Leaf rust	*Puccinia recondita* (early season disease)	24

* data not shown

Control of *Tilletia contraversa*

Dwarf bunt is a significant problem in the Pacific North West region of the USA. No effective and commercially acceptable control of this disease is available to growers, despite the efforts of well funded, exhaustive university and industrial research programs. This lack of effective control measures has lead to a prohibition on export of soft white wheat from this area of the USA to the Peoples Republic of China which has had a zero tolerance for dwarf bunt since 1973.

In 14 Ciba field trials over a five year period (1989 - 1993) difenoconazole gave complete control of dwarf bunt at 12g AI/100kg seed. Table 2 gives a summary of data from 1989 and 1993 which show that even where high levels of disease were present, difenoconazole at 12g AI/100kg seed gave complete control. These data have been confirmed by university and USDA representatives (Goates, 1992; Sitton, 1992; Smiley, 1992).

TABLE 2. % Control of dwarf bunt in the USA using difenoconazole.

Treatment	Rate g AI/100kg seed	Year 1989	1993
untreated	-	(44.9)*	(81.3)**
carboxin+thiram	45 + 45	22.8	19.2
triadimenol	30	22.0	7.5
difenoconazole	12	100	100
number of trials		2	2

* number of infected plants/3.05m row ** % incidence

Control of *Ustilago tritici*

Ustilago tritici has no zero-tolerance requirement in the USA and where control is needed a good certified seed program coupled with high levels of disease control using seed treatments is used.

In 28 trials conducted over six years difenoconazole applied at 12-24 g AI/100kg seed gave an average of 96% control of wheat loose smut. Data from 1990, 1992 and 1993 (Table 3) indicates that there is a slight dose response from 12 - 24g AI/100kg seed. At 12g AI/100kg seed difenoconazole gave more consistent and generally higher levels of control than the standard carboxin+thiram (45+45g AI/100kg seed). Where yield was taken (6 trials) the corresponding yield increase was 10% (data not shown).

TABLE 3. % Control of loose smut on winter wheat using difenoconazole. Mean data from 1990, 1992 and 1993.

Treatment	Rate g AI/100kg	Year 1990	1992	1993
untreated	-	(54.2)*	(45.3)*	(28.3)*
carboxin + thiram	45 + 45	69.5	37.5	94.4
difenoconazole	12	91.7	94.4	95.7
difenoconazole	18	95.0	97.4	98.4
difenoconazole	24	95.0	100	99.6
Number of trials		9	1	3

* number of infected ears/plot. Plot size = $12m^2$

Control of *Tilletia caries*

Both seed-borne and soilborne infections of *T.caries* can be effectively controlled by difenoconazole at 6g AI/100kg (Table 4). Control at this rate has been shown to be consistently high (98.4% control over 15 trials in four years) and equivalent to the best standards.

TABLE 4. % Control of seed and and soil-borne *T. caries* using difenoconazole.

Treatment	Rate g AI/100kg	% infection seed inoculation	soil inoculation
untreated	-	13a	20a
carboxin+thiram	45+45	0b	2.7b
triadimenol	30	0b	0c
difenoconazole	6	0b	0c

Means followed by the same letter are not significantly different according to Duncan's Multiple Range Test, P = 0.05.

Control of early season foliar diseases

Leaf rust (*P. recondita*) is a perennial problem in some parts of the USA. In the USA a seed treatment need only provide protection up to GS30-32 after which foliar fungicides such as propiconazole are used for its control.

In spring (GS30-32) following autumn drilling, difenoconazole (24g AI/100kg seed) will provide good control of leaf rust (Table 5) and this may be extended if disease levels remain moderate.

TABLE 5: % Control of leaf rust on winter wheat using difenoconazole as a seed treatment.

Treatment	Rate g AI/100kg	Trial 306 90	303 90	2 90	36 90
untreated		(9.5)*	(8.3)*	(81.3)*	(80.0)*
triademinol		46.3	67.5	10.8	15.6
difenoconazole		98.6	73.5	38.4	21.9
GS at assessment		32	31-32	31-32	31-32

* % infection in the untreated

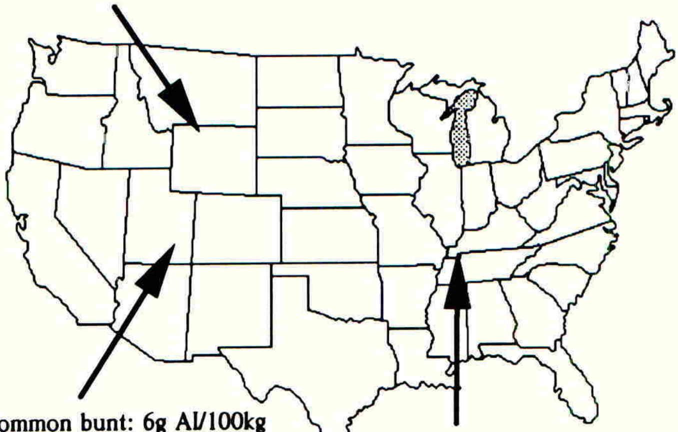
DISCUSSION

Data collected from a wide range of sources show that difenoconazole is able to control all the most important seed-borne diseases of wheat in the USA and gives supression of some foliar pathogens. Especially important is the control of dwarf bunt for which difenoconazole is the first replacement for mercurial seed treatments.

The data also show that difenoconazole has activity against different pathogens at different rates of application (Table 1). In the USA the biological requirements for a seed treatment may differ from region to region. To reflect this difenoconazole will be recommended at a range of rates (6, 12 and 24g AI/100kg seed) depending on the needs of the region and the customer (Figure 2). This allows difenoconazole to be used at the most appropriate dose for any particular situation.

Due to its excellent broad spectrum activity and good crop safety, difenoconazole will be a useful tool for control of wheat diseases in the USA. The consistent improvements in yield and lack of negative seedling growth effects are regarded by officials as important indications that this seed treatment product is likely to be an important factor in disease management on winter wheat (Smiley, 1992).

FIGURE 2. Use of difenoconazole in the USA with respect to rate and region.



Dwarf bunt: 12g AI/100kg
Common bunt: 6g AI/100kg

Common bunt: 6g AI/100kg
Loose smut: 24g AI/100kg
Leaf rust: 24g AI/100kg

Loose smut 24g AI/100kg
Leaf rust 24g AI/100kg

REFERENCES

Dahmen, H.; Staub, T. (1992) Biologogical characterisation and uptake, translocation and dissipation of difenoconazole (CGA169374) in wheat, peanut and tomato plants. *Plant Disease*, 76, 523-526.

Goates, B.J. (1992) Control of dwarf bunt by seed treatment with Dividend 3FS. *Fungicide and Nematicide Tests*, 47, 264.

Ruess, W.; Riebli, P.; Herzog, J.; Speich, J.; James, J.R. (1989) CGA-169374, a new systemic fungicide with novel broad spectrum activity against disease complexes in a wide range of crops. *Proc. Br. Crop Prot. Conf. Pests and Diseases*, 3, 895-900.

Sitton, J.W.; Line, R.F.; Waldher, T.T.; Goates, B.J. (1992) Control of dwarf bunt of wheat with seed treatments. *Fungicide and Nematicide Tests*, 47, 273.

Smiley, R.W. (1992) 1992 Columbia Basin Agric. Res. Report. *Oregon State University Special Report* 894.

Smiley, R.W.; Uddin, W.; Kolding, M. (1992) Control of dwarf bunt with seed and foliar treatments. *Fungicide and Nematicide Tests*, 47, 274.

NEW SEED TREATMENTS BASED ON BITERTANOL AND TEBUCONAZOLE FOR SEED-BORNE DISEASE CONTROL IN WHEAT

D.B. MORRIS, A. WAINWRIGHT, R.H. MEREDITH

Bayer plc, Crop Protection Business Group, Elm Farm Development Station,
Great Green, Thurston, Bury St Edmunds, Suffolk, IP31 3SJ

ABSTRACT

In trials conducted during 1988-93 on wheat the seed treatments bitertanol/fuberidazole and bitertanol/triadimenol/fuberidazole gave excellent control of bunt and of seedling blight particularly when caused by *Monographella nivalis*. Tebuconazole was effective against loose smut and both seed and soil-borne bunt, but combinations of tebuconazole with guazatine or thiram were necessary to extend the disease control spectrum to include seedling blights. These treatments offered significant improvements over modern commercial standards.

INTRODUCTION

Since the withdrawal of organomercurial seed treatments in the UK, a renewed interest has been shown in the control of seed-borne cereal pathogens and the performance of new active ingredients. This paper reports on wheat trials conducted over 6 seasons from 1988-93 with seed treatments based on bitertanol or tebuconazole. The results of trials against seed-borne diseases of barley are also reported in these proceedings (Wainwright *et al.*, 1994). Bitertanol is a broad-spectrum triazole fungicide with limited systemic activity which was first developed for application to pome and bush fruit (Birch *et al.*, 1981). Tebuconazole, also a triazole fungicide, has been developed in the UK principally for use in cereal and oilseed rape crops, and initial results obtained with spray and seed treatment formulations were reported by Wainwright & Linke (1987). Early trials with tebuconazole on wheat identified the need for an additional AI against Fusarium and applications with guazatine and thiram were included in subsequent work.

Most trials were handsown into small plots at Bayer's Elm Farm Development Station, Suffolk which enabled a large number of treatments to be tested under controlled conditions. Further experience against some of the more important pathogens was gained in 1992/3 by machine drilling trials at various sites throughout the UK.

METHODS AND MATERIALS

The treatments, which were applied as liquid or flowable formulations, are listed in Table 1. Application was by hand using glass jars for handsown trials and by mini-Rotostat for machine drilled trials. Trials were fully replicated using randomised block designs, and plot sizes were 1 m x 1 m (hand sown) and 4 m x 1.5 m (Oyjord-drilled). Naturally infected seed was used with the exception of bunt which was artificially inoculated by dusting healthy seed with fungal spores (1 or 2 g spores per kg) or by inoculating soil using 0.5 or 1 g spores in 100 g sand per m² plot. Sowing rates were 25 or 40 g/m² plot depending on the level of seed infection and 160 kg/ha for Oyjord drilled trials.

TABLE 1. Formulations included in trials and rates of application.

Product	Active ingredients	Application rate per 100 kg seed ml	g AI
Exp.1	bitertanol/fuberidazole	150	56.25/3.45
Exp.2	bitertanol/triadimenol/fuberidazole	200	37.6/15/4.6
Exp.3	tebuconazole	120	3
Exp.4	tebuconazole	200	3
Exp.6	tebuconazole/guazatine	200	3/60
Exp.7	tebuconazole/guazatine	200	3/60
Exp.8	tebuconazole/thiram	200	3/100
Baytan® flowable	triadimenol/fuberidazole	200	37.5/4.5
Ceresol®	phenyl mercury acetate (PMA)	110	3.7
Cerevax®	carboxin/thiabendazole (TBZ)	250	90/5
Rappor®	guazatine	200	60
Vincit® L	flutriafol/thiabendazole (TBZ)	200	5/5

Sixteen hand-sown trials were located at Elm Farm Development Station:

Winter wheat -	seedling blight (*Monographella nivalis*)	6 trials
	seedling blight (*Fusarium culmorum*)	1 trial
	seedling blight (*Leptosphaeria (Septoria) nodorum)*	1 trial
	loose smut (*Ustilago nuda*)	2 trials
	bunt (*Tilletia caries*) (including 1 soil, 1 seed + soil-borne)	5 trials
Spring wheat -	bunt (*T. caries*)	1 trial

The handsown trials were generally sown late, ie November, under conditions favouring seedling blights and bunt infection.

Two winter wheat cultivars infected with *M. nivalis* were drilled at each of 4 sites (Norfolk, Glos., Lincs., and E. Lothian) using an Oyjord plot drill in 1992/3 and a further 2 sites (Suffolk and Warks.) were drilled with bunt infected seed.

Trials were assessed by counting diseased and healthy plants or ears in each plot to obtain percent control/reduction figures. Stem base browning symptoms on seedlings were graded and an index based on disease severity was calculated (Table 3). Bunt and loose smut were assessed at GS 65-85 whilst seedling diseases were assessed at GS 12-13. Plant stand was recorded at all sites to monitor crop safety and the effect of seedling blights.

RESULTS

The results of trials are summarised in Tables 2-5. Crop stand counts reflect disease control in seedling blight trials and are given in Table 2. The results of stem base browning assessments in these trials are given in Table 3.

TABLE 2. Relative emergence (%) in seedling disease m² trials.

Treatments	*Monographella nivalis*						*Fusarium culmorum*	*Septoria nodorum*
	FA/06 1988	FA/03 1989	FA/06 1990	FA/05 1991	FA/05 1992	FA/06 1993	FA/06 1992	FA/07 1990
bit./fub.		208	109	978	659	200	191	109
bit./triad./fub.		216	129	983	637	185	188	109
tebuconazole	306	167	107	196	185	129	189	96
tebucon./guaz.	896	198	119	974	652	177		101
tebucon./thiram				980	489	224	209	
triad./fub.	782	209	126	733	652	201	205	100
PMA	827	212	121	910				100
carboxin/TBZ				580	544	193	194	
guazatine				1058	587	216	199	
flutria./TBZ					178	104	197	
untreated (plants/m²)	(34)	(143)	(312)	(41)	(56)	(162)	(242)	(391)

TABLE 3. Reduction in stem infection (%) in m² trials.

Treatments	*Monographella nivalis*						*Fusarium culmorum*	*Septoria nodorum*
	FA/06 1988	FA/03 1989	FA/06 1990	FA/05 1991	FA/05 1992	FA/06 1993	FA/06 1992	FA/07 1990
bit./fub.		100	96	74	89	92	89	96
bit./triad./fub.		100	100	93	98	91	95	100
tebuconazole	38	35	75	0	0	50	52	96
tebucon./guaz.	93	95	100	96	83	86		100
tebucon./thiram				77	28	88	89	
triad./fub.	64	92	91	19	30	47	92	100
PMA	68	60	37	24				99
carboxin/TBZ				0	2	18	87	
guazatine				93	74	84	23	
flutria./TBZ					0	23	56	
untreated (% infection index)	(26)	(44)	(26)	(39)	(60)	(22)	(10)	(31)

In trials that were free of seedling diseases, small reductions in crop stand and delayed emergence were usually seen with triazole treatments, though this was not observed with bitertanol/fuberidazole.

A particularly high level of bunt, 62 % infection, was recorded in FA/06 1991 (Table 4) which demonstrated the potential of soil-borne inoculum. Only 2 trials were carried out against loose smut due to limited availability of seed and complete control was achieved with all treatments containing a triazole active ingredient or carboxin, though the same seed batch had to be used in both trials.

TABLE 4. Reduction in bunt and smut infection (%) in m^2 trials.

Treatments	*Tilletia caries*						*Ustilago nuda*	
			(so)	(s/s)		(sp.)		
	FA/07 1988	FA/08 1990	FA/06 1991	FA/08 1992	FA/08 1993	FA/23 1991	FA/07 1992	FA/07 1993
bit./fub.		100	100	100	100	100	100	100
bit./triad./fub.		100	100*	100*	100	100*	100*	100*
tebuconazole	99	99	100	100	93	100	100	100
tebucon./guaz.	100	100	100					
tebucon./thiram				99	98		100	100
triad./fub.	100	100	100	100	100	100	100	100
PMA		100	32					
carboxin/TBZ			54	46	87		100	100
guazatine			34	0	82	92	4	13
flutria./TBZ				100	96		100	
untreated (% infected ears)	(24)	(37)	(62)	(24)	(22)	(20)	(3)	(3)

* - rate 0.75 normal (so) - soil inoculated (s/s) - seed/soil inoculated
(sp.) - spring wheat

The meaned results from Oyjord drilled trials against seedling blight and bunt are summarised in Table 5. Although disease expression varied slightly from site to site, treatment performance was similar to that observed in handsown plots.

All data were statistically analysed but lack of space prevents the inclusion of this information.

TABLE 5. Disease control in Oyjord drilled trials 1992-1993.

Treatments	*M. nivalis* - 4 sites				*T. caries* - 2 sites
	% Rel emergence		% Redn infection		% Redn infection
	Haven	Riband	Haven	Riband	Beaver
bit./fub.	226	217	87	84	100
bit./triad./fub.	221	237	92	84	100
tebuconazole	171	161	25	20	99
tebucon./thiram	233	226	64	58	100
triad./fub.	224	232	64	33	100
guazatine	229	248	83	70	90
untreated	(22)	(21)	(52)	(50)	(53)
	(plants/m row)		(% index)		(% infected ears)

DISCUSSION

Until their withdrawal from use in 1992, the organomercury seed treatments provided cheap and effective protection against seedling blights caused by *Fusarium* spp. and bunt which are generally considered the most important seed-borne diseases of wheat.

Good crop establishment may be jeopardised by seedling blights particularly following a wet summer when seed infection is likely to be high or when seed is drilled late. During six years of trials in the UK, bitertanol based treatments consistently resulted in excellent crop stands which were comparable to the best standards especially where *M. nivalis* was present on the seed. In addition, these treatments gave the greatest reductions in post-emergence stem browning symptoms. Tebuconazole alone was not effective in controlling high levels of seedling blight and a combination with guazatine or thiram was required.

It is essential to prevent bunt infection because of its ability to build up rapidly in untreated seed stocks and to spread via soil inoculum, the latter being cited as the cause of some recent outbreaks (Yarham, 1993). Soil-borne bunt was only controlled well by triazole treatments; bitertanol was surprisingly effective considering its limited sytemicity. Seed-borne bunt was largely well controlled except in 1993 when treatments were less reliable and carboxin and guazatine gave less than 90 % control.

Loose smut, a relatively minor disease in wheat, was completely controlled by all triazole treatments and carboxin in these trials. Some earlier results had indicated that bitertanol may not give consistent control of loose smut and this led to the alternative co-formulation of bitertanol which included triadimenol.

Against the range of seed-borne diseases of wheat, the seed treatments bitertanol/fuberidazole, bitertanol/triadimenol/fuberidazole, tebuconazole/guazatine and tebuconazole/thiram, offer a significant improvement over organomercury and modern commercial standards.

ACKNOWLEDGEMENTS

The authors would like to thank colleagues who have contributed to this work.

REFERENCES

Birch, P.A.; Rose, P.W.; Wainwright, A. (1981) A broad spectrum fungicide of the triazole group for use in pome and bush fruit. *British Crop Protection Conference - Pests and Diseases 1981*, **2**, 545-554.

Wainwright, A.; Linke, W. (1987) Field trials with Folicur® and Raxil® against foliar and seedborne cereal diseases, and observations on the control of rape diseases with Folicur in Great Britain (1984-1986). *Pflanzenschutz-Nachrichten Bayer,* **40**, 181-212 (English edition).

Wainwright, A.; Morris, D.B.; Meredith, R.H. (1994) New seed treatments based on bitertanol, tebuconazole and triazoxide for seed-borne disease control in barley. *These proceedings*.

Yarham, D.J. (1993) Soil-borne spores as a source of inoculum for wheat bunt (*Tilletia caries*). *Plant Pathology*, **42**, 654-656.

NEW SEED TREATMENTS BASED ON BITERTANOL, TEBUCONAZOLE AND TRIAZOXIDE FOR SEED BORNE DISEASE CONTROL IN BARLEY

A. WAINWRIGHT, D.B. MORRIS, R.H. MEREDITH

Bayer plc, Crop Protection Business Group, Elm Farm Development Station,
Great Green, Thurston, Bury St Edmunds, Suffolk, IP31 3SJ

ABSTRACT

In trials on barley between 1988-1993, a seed treatment formulation containing bitertanol/triadimenol/fuberidazole was effective against loose smut, covered smut, foot rot (*Cochliobolus*), leaf stripe in spring barley and organomercury resistant leaf stripe in winter barley. Compared with triadimenol/fuberidazole, there was some improvement against leaf stripe in winter barley (organomercury sensitive), net blotch, and stem browning caused by *Monographella nivalis*.

Tebuconazole gave good control of loose smut and covered smut and was also active against leaf stripe, net blotch and foot rot (*Cochliobolus*). Co-formulation of tebuconazole with imazalil or triazoxide improved performance. Triazoxide showed outstanding activity against *Pyrenophora* spp. and in combination with tebuconazole provides a wide-spectrum treatment for barley.

INTRODUCTION

This paper reports on barley trials carried out over six seasons, 1988-1993, with seed treatments based on bitertanol or tebuconazole. Trials against seed-borne diseases of wheat are reported elsewhere in these proceedings by Morris *et al.* (1994). In discussing early results with tebuconazole on barley, Wainwright & Linke (1987) suggested the addition of a specific AI for reliable control of leaf stripe. Subsequent trials included treatments containing either imazalil or triazoxide, a new benzotriazine fungicide which is also highly specific against *Pyrenophora* spp. (Dutzmann, 1994).

Most trials were carried out as handsown plots at Elm Farm Development Station in Suffolk whilst in 1992/3 machine drilled trials were located at various sites throughout the UK.

METHODS AND MATERIALS

The treatments, which were applied as liquid or flowable formulations, are listed in Table 1. Application was by hand using glass jars for handsown trials and by mini-Rotostat for machine drilled trials. Trials were fully replicated using randomised block designs and plot sizes were 1 m x 1 m (hand sown) and 4 m x 1.5 m (Oyjord-drilled). Naturally infected seed was used with the exception of covered smut for FA/05, 1993 which was artificially inoculated using a spore suspension in water. Sowing rates were 25 or 40 g/m^2 plot depending on the level of seed infection and 140 kg/ha for Oyjord drilled trials.

TABLE 1. Formulations included in trials and rates of application.

Product	Active ingredients	Application rate per 100 kg seed	
		ml	g AI
Exp.2	bitertanol/triadimenol/fuberidazole	200	37.6/15/4.6
Exp.3	tebuconazole	120	3
Exp.4	tebuconazole	200	3
Exp.5	tebuconazole/triazoxide	150	3/3
Exp.6	tebuconazole/imazalil	200	3/4
Baytan® flowable	triadimenol/fuberidazole	200	37.5/4.5
Ceresol®	phenyl mercury acetate (PMA)	110	3.7
Cerevax® Extra	carboxin/thiabendazole (TBZ)/imazalil	200	60/5/4
Murganic® RPB	carboxin/phenyl mercury acetate	220	79.2/3.7
Rappor® Plus	guazatine/imazalil	220	66/5.5

Forty four trials were handsown at Elm Farm Development Station:

		winter/spring trials
Barley:	seedling blight (*Monographella nivalis*)	2^{+}/ 6
	net blotch (*Pyrenophora teres*)	1 / 1
	foot rot (*Cochliobolus sativus*)	- / 5
	leaf stripe (*Pyrenophora graminea*)	9*/ 7
	loose smut (*Ustilago nuda*)	5 / 6
	covered smut (*Ustilago hordei*)	2 / -

* organomercury sensitive/resistant = 5/4

\+ One trial was a spring cultivar sown in the autumn.

Four winter barley cultivars, two infected with leaf stripe and two with loose smut were drilled at each of four sites (Norfolk, Lincs., Yorks., and Kent) using an Oyjord plot drill in 1992/3.

Trials were assessed by counting diseased and healthy plants or ears in each plot to obtain percent control/reduction figures. Stem base browning symptoms on seedlings were graded and an index based on disease severity was calculated. Plant stand was recorded at all sites to monitor crop safety and the effect of seedling blights.

RESULTS

The results of individual hand-sown trials are given in Tables 2-7. Space does not allow the inclusion of crop stand data which was recorded in all trials; any reduced or delayed emergence was as normally expected in seed treatments with a triazole component. Seedling blight in barley trials was not severe even when seed was heavily infected with *M. nivalis*, and little benefit in terms of increased crop stand was obtained from seed treatments.

TABLE 2. Reduction in stem infection (%) in m^2 trials.

Treatments	*Monographella nivalis*							
	W. barley		S. barley					
	FA/01 1988	FA/02# 1993	FA/22 1988	FA/17 1989	FA/20 1990	FA/19 1991	FA/17 1992	FA/21 1993
bit./triad./fub.		92		100	98	94	92	95
tebuconazole	0	0	0	92	96	49	7	20
tebucon./triaz.	0		0		94		31	20
tebucon./imaz.							9	24
triad./fub.	80	54	16	99	98	82	59	53
PMA	64		44	36	92	79		
carbox./TBZ/imaz.		78				81*	30	43
guazatine/imaz.		87				91	73	80
untreated (% infection index)	(12)	(9)	(62)	(17)	(10)	(7)	(19)	(32)

* - rate 1.25 normal # - spring cultivar sown in autumn

TABLE 3. Reduction in seedling infection (%) in m^2 trials.

Treatments	*Pyrenophora teres*		*Cochliobolus sativus*				
	W. barley	S. barley	S. barley				
	FA/03 1990	FA/23 1993	FA/20 1989	FA/21 1990	FA/20 1991	FA/18 1992	FA/22 1993
bit./triad./fub.	97	98	94	99	91	74	87
tebuconazole	72	73	48	97	64	68	72
tebucon./triaz.	94	100	73	90		75	63
tebucon./imaz.		96				83	79
triad./fub.	97	62	87	94	88	88	76
PMA	100		36	66	36		
carbox./TBZ/imaz.		98			90*	74	78
guazatine/imaz.		99			83	84	75
untreated	(2)	(12)	(33)	(42)	(38)	(33)	(28)
	(% infected plants)		(% infection index)				

* - rate 1.25 normal

Only 0.3 % covered smut was recorded in untreated plots in FA/04, 1992. In the following year an increased level of 1.3 % smutted ears was obtained with artificially inoculated seed - see Table 6.

The meaned results from Oyjord drilled trials against leaf stripe and loose smut are summarised in Table 8.

Statistical information is not included due to lack of space.

TABLE 4. Reduction in leaf stripe infection (%) in W. barley m² trials.

Treatments	*Pyrenophora graminea* (PMA resistant strain)				*Pyrenophora graminea* (PMA sensitive strain)				
	FA/02 1988	FA/04 1989	FA/04 1990	FA/03 1991	FA/03 1988	FA/05 1989	FA/02 1991	FA/02 1992	FA/03 1993
bit./triad./fub.		100	100	100		96	97	83	65
tebuconazole	100		100	100	95		85	86	30
tebucon./triaz.	100		100		100				100
tebucon./imaz.				100			97	98	72
triad./fub.	100	100	100	100	30	32	4	12	0
PMA	56	42	58	87	100	100	99		
carbox./TBZ/imaz.				100			97	99	49
guazatine/imaz.				100			99	100	80
untreated (% infected tillers)	(0.5)	(3.3)	(3.2)	(1.1)	(19)	(21)	(28)	(36)	(33)

TABLE 5. Reduction in leaf stripe infection (%) in S. barley m² trials.

Treatments	*Pyrenophora graminea*						
	FA/23 1988	FA/18 1989	FA/22 1990	FA/21 1991	FA/22 1991	FA/19 1992	FA/24 1993
bit./triad./fub.		100	95	98	100	100	100
tebuconazole	84	87	83	85	95	100	99
tebucon./triaz.	100	100	100			100	100
tebucon./imaz.				96	100	100	100
triad./fub.	100	100	100	98	100	100	100
PMA	47	42	0	0	0		
carbox./TBZ/imaz.				94*	100*	100	100
guazatine/imaz.				99	100	99	100
untreated (% infected tillers)	(3.0)	(9.0)	(1.3)	(6.7)	(1.5)	(5.8)	(17.7)

* - rate 1.25 normal

DISCUSSION

Leaf stripe and loose smut are generally considered to be the most important of the seed-borne diseases of barley. Seed treatments which contained organomercury alone were able to give cheap, effective control of leaf stripe and other diseases whilst carboxin could be added to extend the range to include loose smut. However, during the decade before the withdrawal of mercury, resistance in *P. graminea* was identified in the field (Locke, 1986) and clearly there was a requirement for new active compounds.

TABLE 6. Reduction in smut infection (%) in W. barley m^2 trials.

Treatments	*Ustilago nuda*					*Ustilago hordei*	
	FA/04	FA/05	FA/04	FA/03	FA/04	FA/04	FA/05
	1988	1990	1991	1992	1993	1992	1993
bit./triad./fub.		100	100*	100*	100*	100*	100*
tebuconazole	100	99	100	100	100	100	100
tebucon./triaz.	100	100			100		
tebucon./imaz.					99		
triad./fub.	100	100	100	100	100	100	100
carboxin/PMA	100	98					
carbox./TBZ/imaz.			97	98	100	100	100
guazatine/imaz.			67	48	69	100	100
untreated (% infected tillers)	(2.6)	(9.2)	(4.9)	(10.8)	(10.2)	(0.3)	(1.3)

* - rate 0.75 normal

TABLE 7. Reduction in smut infection (%) in S. barley m^2 trials.

Treatments	*Ustilago nuda*					
	FA/18	FA/21	FA/23	FA/22	FA/20	FA/25
	1989	1989	1990	1991	1992	1993
bit./triad./fub.	100	99	100	100	98*	87*
tebuconazole	100	92	86	100	90	91
tebucon./triaz.	100	100	100		93	99
tebucon./imaz.				100	91^	99
triad./fub.	100	100	100	100	99	100
carboxin/PMA		95	98			
carbox./TBZ/imaz.				98#	92	75
guazatine/imaz.				7	0	0
untreated (% infected tillers)	(0.4)	(2.2)	(1.6)	(14.3)	(5.4)	(3.8)

* - rate 0.75 normal   # - rate 1.25 normal   ^ - rate 0.67 normal

Whilst all treatments gave complete control of organomercury resistant leaf stripe on winter barley, the partial substitution of triadimenol with bitertanol led to an improvement over triadimenol/fuberidazole against the sensitive strain (Tables 4 and 5). The addition of imazalil or triazoxide to tebuconazole raised performance against leaf stripe although only the triazoxide combination was completely effective in 1993.

The seedling diseases including Fusarium (*M. nivalis*) and footrot (*C. sativus*) are usually of minor importance during the establishment of a barley crop. Against these diseases and net blotch, bitertanol conferred

TABLE 8. Disease control in Oyjord drilled trials.

Treatments	*Pyrenophora graminea* - mean of 4 sites		*Ustilago nuda* - mean of 4 sites	
	Gaulois	Flamenco	Pastoral	Panda
bit./triad./fub.	81	66	100	100
tebuconazole	85	68	100	100
tebucon./triaz.	100	100	100	100
tebucon./imaz.	99	98	100	100
triad./fub.	12	7	100	100
untreated (% infected tillers)	(14)	(22)	(8)	(7)

additional benefits over triadimenol/fuberidazole. The performance of tebuconazole against net blotch on the heavily infected cultivar Peel was clearly enhanced by the addition of imazalil and particularly triazoxide.

Tebuconazole and triadimenol formulations were very effective against loose smut, though the reliability of tebuconazole in spring barley was improved in formulations with imazalil or triazoxide. In handsown trials in 1992-93 (Table 7), reduced control may be associated with late sowing during warm, rapid-growing conditions when it is known that triazole performance can be impaired (Martin & Edgington, 1980).

For control of the range of seed-borne diseases in barley, bitertanol/triadimenol/fuberidazole, tebuconazole/imazalil and tebuconazole/triazoxide offer improvements over existing standards. In recent years a heavy reliance has been placed on imazalil for leaf stripe control by its inclusion in a number of commercial formulations; the different chemistry and excellent efficacy of triazoxide should therefore make it a valuable addition to the limited treatment range currently available.

REFERENCES

Dutzmann, S. (1994) Triazoxide - a new seed treatment fungicide for the control of seed borne *Pyrenophora* spp. *These proceedings*.

Locke, T. (1986) Current incidence in the United Kingdom of fungicide resistance in pathogens of cereals. *British Crop Protection Conference - Pests and Diseases 1986*, **2**, 781-786.

Martin, R.A.; Edgington, L.V. (1980) Effect of temperature on efficacy of triadimenol and fenapanil to control loose smut of barley. *Canadian Journal of Plant Pathology*, **2**, 201-204.

Morris, D.B.; Wainwright, A.; Meredith, R.H. (1994) New seed treatments based on bitertanol and tebuconazole for seed-borne disease control in wheat. *These proceedings*.

Wainwright, A.; Linke, W. (1987) Field trials with Folicur® and Raxil® against foliar and seed-borne cereal diseases, and observations on the control of rape diseases with Folicur in Great Britain (1984-1986). *Pflanzenschutz-Nachrichten Bayer*, **40**, 181-212 (English edition).

COST BENEFIT ANALYSIS OF RUSSIAN WHEAT APHID (*DIURAPHIS NOXIA*) CONTROL IN SOUTH AFRICA USING A SEED TREATMENT.

M.C. VAN DER WESTHUIZEN, J. DE JAGER

Department of Zoology and Entomology, University of the Orange Free State, Bloemfontein, 9300, South Africa.

M.H. MASON, M.W. DEALL

Bayer (Pty) Ltd., Isando, 1600, South Africa.

ABSTRACT

The cost and control effectiveness of imidacloprid as the active ingredient of a seed dressing was evaluated under dry land condition in the suppression of *Diuraphis noxia* on wheat. The optimum dosage of 117 g AI or 167 g product per (deca tonne) seed provided an investment return of 4.23. The registered rate of 200 g product of Gaucho 70 WS per dt seed provides effective control of the aphid and is economically justified.

INTRODUCTION

The Russian Wheat Aphid (RWA) (*Diuraphis noxia*) is currently the most important pest of wheat in South Africa. The RWA was first recorded in South Africa in 1978 and within a few years had spread to all the wheat-producing areas in the country (Aalbersberg *et al.*, 1987). However, its major economic impact is on the wheat of the Orange Free State. One million hectares of wheat are grown in the Orange Free State, which accounts for approximately 60% of South Africa's total crop. The average yield for this region is 1.5 ton/ha. Measurements of single plant yields have shown that a reduction in yield of up to 90% can occur as a result of RWA infestations (Du Toit & Walters, 1984). The loss from RWA is estimated at between US $16 and 18 million annually.

The Orange Free State receives rainfall during the summer months, which is preserved by appropriate cultivation practices for subsequent planting of wheat in autumn. The low humidity and virtually no rainfall during the growing season favours the development of large populations of this species.

Characteristic symptoms of RWA infestation are white, reddish-purple and/or yellow streaking of infested leaves as well as inward curling of the leaf edges. The latter leads to a reduction in the photosynthetic area of the leaf. The aphids and the typical symptoms are mainly found on the new growth and in the later growth stages tend to occur on the flag leaf. Aphid colonisation may result in damage to the ears which become bent and turn white, with consequent incomplete filling of the ear (Aalbersberg, 1987).

Control strategies utilising host genetic resistance, chemical and biological methods have been used in South Africa and other areas of the world to control RWA.

Traditionally, a wide range of insecticides have been used to control RWA and in 1991 RWA resistant cultivars were introduced in South Africa for the first time. Recently an insecticide seed dressing, Gaucho 70 WS (imidacloprid), was introduced and the objective of this study was to establish guidelines for the wheat industry on the economic effectiveness of the compound in controlling RWA.

The trials were done to determine a) the influence of various dosage rates of imidacloprid on the aphid infestation rate of the crops under field conditions, b) the period of control and type of aphid suppression (tolerance or antibiosis) and c) the economic response to control measures by means of the various response curves:

- Pest infestation levels
- Cultivar growth stage response
- Cost benefit analysis
- Crop response

MATERIAL AND METHODS

The trials were conducted at the Glen Agricultural Development Institute in the Western Orange Free State. A randomised block design with five replicates was used. The plots were 2.7 m wide, consisting of six wheat rows and 10 m long. The outer row on either side was used as an infestation source and only the inner four rows were used for evaluations. Seeding was done with a Gaspardo precision planter, planting six rows, using the cultivar Scheepers '69' at a seed density of 18 kg/ha.

Treatments included an aphid free control, obtained by regular foliar insecticide application throughout the growing period and seed dressing with imidacloprid at 0, 35, 70, 105, 140 and 175 g AI/dt seed.

The following parameters for the various treatments were recorded weekly: date of infestation, number of tillers infested and growth stage (Zadoks *et al.*, 1974). Infested tillers were labelled weekly with various coloured tags indicating date (growth stage) of infestation. All infested tillers were individually harvested throughout the entire plot, while the tillers not infested were collectively yielded. Plot yields were expressed as the summed mass of these treatments. The yield and infestation parameters of the various treatments were analysed by analysis of variance and the means compared using the Student-Newman-Keuls test (Steel & Torrie, 1960). Zar's method of multiple regression analysis was used to compare slopes and elevations (Y-intercepts) of linear regressions (Zar, 1974).

The following economics of chemical control and wheat production were used in the cost benefit analysis of seed dressing as well as the calculation of economic damage and economic injury levels for foliar insecticide applications (tractor and aerial) and seed dressing:

Cost of foliar aphicide per ha	US $ 8.38
Tractor application per ha	US $ 2.55
Aerial application per ha	US $ 6.61
Gaucho 70 WS per kg	US $ 212.78
Wheat price per ton	US $ 181.38

The cost benefit analysis was based on the procedures of Flint & Van den Berg (1981) and Van der Westhuizen (1992) and the procedure of Chiang (1973) and Van der Westhuizen (1992) were used in the calculations of economic damage and economic injury levels for cereals.

RESULTS AND DISCUSSION

RWA infestation in the absence of chemical control

Fig. 1 shows the normal pattern of horizontal infestation (among tillers) of Scheepers '69' by the RWA in the absence of any chemical control measures. The percentage tillers infested at the various dates of infestation after planting was based on average infestation of the five plots. An exponential growth rate of 0.4563 percent tillers infested per day occurred in the absence of chemical control.

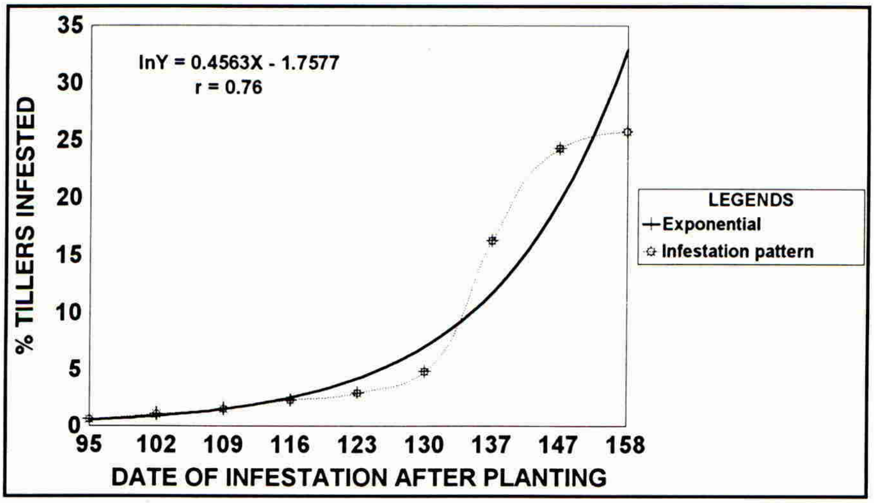


Fig. 1. Horizontal infestation of cultivar Scheepers '69' by RWA.

Impact of RWA on yield in the absence of chemical control

The yield of tillers infested and not infested from plants that were infested and not infested are shown in Table 1. No significant difference in yield occurred between tillers not infested from plants that were either infested or not infested ($F = 0.58$; $p > 0.05$). There was a significant difference in yield however, between infested tillers and tillers not infested ($F = 310.01$; $p < 0.01$). These results indicated that the effect on yield by the RWA feeding stays within the tiller of infestation and no reduction in yield of adjacent non infested tillers within the same plant occurred.

TABLE 1. Inter- and intra tiller effect of RWA feeding on yield of tillers (g).

	INFESTATION STATUS	
TILLERS	PLANTS	
RWA	Infested	Not Infested
Infested	0.553	-
Not Infested	0.917	0.935

The effect of RWA infestation at various growth stages of the cultivar Scheepers '69' with regard to reduction in yield per tiller is shown in Table 2. The growth stage response identifies the most sensitive growth stage of specific cultivars to RWA infestation. According to Table 2 the greatest impact of RWA occurs when infestation takes place at growth stage 57. Thus, the economic injury level should be based on the impact of varying infestation levels of RWA at approximately growth stage 60 of the cultivar Scheepers '69'.

TABLE 2. Percentage reduction in yield per tiller infested at various dates and growth stages of the cultivar Scheepers '69'.

	DATE OF INFESTATION AFTER PLANTING								
	95	102	109	116	123	130	137	147	158
Growth stage (Zadoks)	30	32	33	34	37	38	43	57	69
Reduction in yield (%)	2.2	1.8	2.5	2.4	8.0	9.2	34.9	44.8	3.7

RWA infestation in the presence of chemical control

The effect of various dosage rates of imidacloprid on the infestation pattern of the cultivar Scheepers '69' is represented in Fig. 2. The seed dressing resulted in a significant reduction in numbers of tillers infested ($F = 16.38$; $p < 0.01$).

The exponential growth of the infestation patterns achieved with the various dosages of seed treatments were determined and are shown in Table 3. From this table it is clear that imidacloprid treatments did not affect the infestation rate ($F = 2. 13$; $p > 0.05$) of RWA, but resulted in a significant decrease in the Y-intercepts of these growth curves ($F = 4.45$; $p < 0.05$). Thus, according to these results, imidacloprid provides no suppression in infestation rate (antibiosis), but does, however, provide an increased period of total control of RWA infestation.

Stimulatory effect of imidacloprid

The relationship between various dosages of imidacloprid and yields of tillers not infested showed a tendency of increasing yield ($Y = 0.9292 + 9.1429 \times 10^{-5}X$, $r = 0.4180$). These increases are however not significant ($F = 1.62$; $p > 0.05$).

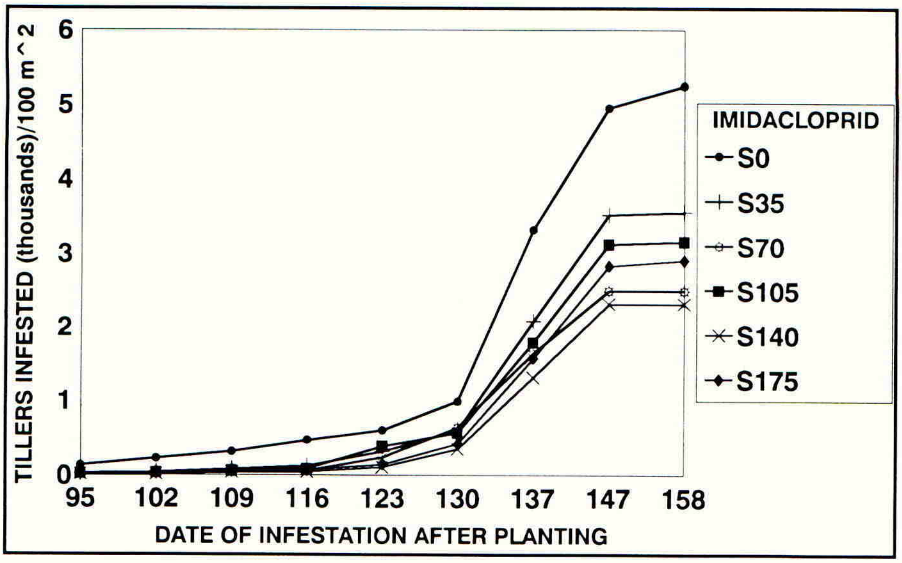


Fig. 2. Effect of various dosages of imidacloprid seed dressing (g AI/dt seed) on tillers infested with RWA.

TABLE 3. Impact of various dosage rates of imidacloprid on the growth of RWA with regard to percentage tillers infested per day.

Imidacloprid dosage g/dt seed	Exponential growth rate/day % tillers infested	Y-intercept
0	0.4563	-1.7577
35	0.4180	-2.7074
70	0.4914	-3.0506
105	0.4434	-3.0150
140	0.4482	-3.5323
175	0.4862	-3.3832

Impact of RWA on yield in the presence of chemical control

The effect of imidacloprid seed dressing on the mean infestation level and yields of the plots treated with various dosages is shown in Table 4. Seed dressing as a component of variance resulted in a significant decrease in final infestation ($F = 16.38$; $p < 0.01$) and increase in yield ($F = 48.24$; $p < 0.01$). The 140 g AI per dt seed treatment rate gave the best control of RWA. The highest efficacy in control and yield response lies between dosage rates of 70 and 140 g AI per dt seed.

TABLE 4. Effect of various dosages of imidacloprid on mean infestation levels and yield of cultivar Scheepers '69'.

Imidacloprid dosage g/dt seed	RWA infestation % tillers	Yield ton/ha
0	25.8	1.667
35	17.4	1.800
70	12.2	1.825
105	15.5	1.850
140	11.3	1.830
175	14.2	1.857

Economic implications of chemical control

Theoretically, as the damage done by RWA decreases, the value of the crop increases up to a point where the pest population reaches zero. The increase to maximum value of the crop may not necessarily be justified by the cost of the control measures used. Hypothetically, the highest profit or cost benefit is the greatest difference between production advantage and cost of control. Fig. 3 represents the cost benefit analysis for the various treatments evaluated in the trials. The cost of control was based on the amount of product used per ha. The investment return indices (IRF index) were calculated as follows:

$$\frac{(\text{Control benefit - Control cost})^3}{100}$$

Peak indices coincide with maximum return in control (Van der Westhuizen, 1992). The cost benefit analysis indicates an optimum dosage of 167 g product per dt seed with an investment return of 4.23.

The pest crop response for Scheepers '69' at the most sensitive growth stage, GS 57 (see Table 2), is shown in Fig. 4. The economic injury levels (EIL) of tractor and aerial applications of a foliar spray is indicated on the figure. Furthermore, the actual infestation of the tillers in the untreated control (S0) and the plots planted with 140 g AI imidacloprid per dt seed (S140) are indicated.

From the crop pest response curve (Fig. 4) as well as the actual RWA infestations in the untreated control, foliar application (tractor and aerial) would have been justified. However, at the seed treatment rate of 140 g AI/dt, RWA was sufficiently suppressed, so that no further corrective treatment would have been justified.

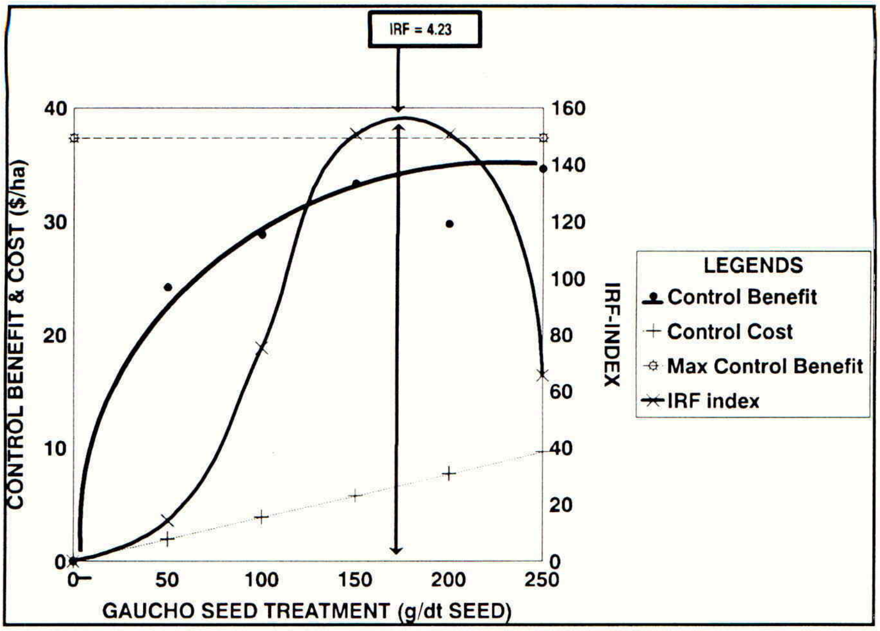


Fig. 3. Cost benefit analysis of imidacloprid seed dressing in the chemical control of RWA as pest of the wheat cultivar Scheepers '69'.

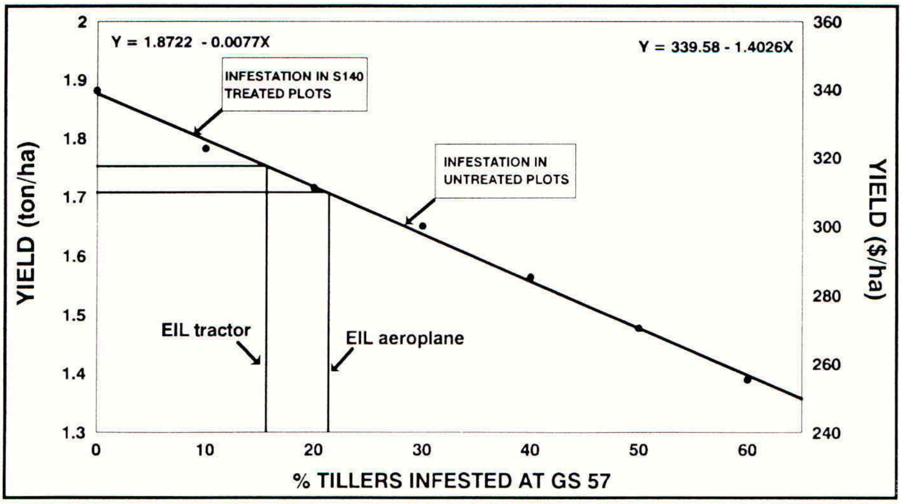


Fig. 4. Yield response of the cultivar Scheepers '69' with increasing levels of RWA infestation as well as the guidelines for corrective tractor and aerial applications of foliar aphicides.

It can be concluded that the South African registered rate of 200 g product of Gaucho 70 WS per dt of seed (140 g AI) is close to the optimum dosage of 167 g (117 g AI) (Fig. 3) and provides effective and economically justifiable control of RWA.

ACKNOWLEDGEMENTS

Financial support was provided by Bayer (Pty) Ltd., Isando, Department of Agriculture, Glen and the University of the Orange Free State. Messrs. J.R. Richter, P. Aldrich and Mrs. D. van der Merwe assisted with the research.

REFERENCES

Aalbersberg, Y.K. (1987) *Ecology of the wheat aphid* Diuraphis noxia *(Mordvilko) in the Eastern Orange Free State.* M.Sc. thesis, University of the O.F.S., Bloemfontein.

Aalbersberg, Y.K.; Van der Westhuizen, M.C.; Hewitt, P.H. (1987) A simple key for the diagnosis of the instars of the Russian wheat aphid, Diuraphis noxia (Mordvilko) (Hemiptera: Aphididae). *Bulletin of Entomological Research* **77**, 637-640.

Chiang, H.C. (1973) Ecological considerations in developing recommendations for chemical control of pests: European corn borer as a model. *FAO Plant protection Bulletin* **21**, 30-39.

Du Toit, F.; Walters, M.C. (1984) Damage assessment and economic threshold values for the chemical control of the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) on winter wheat. *Technical Communication of the Department of Agriculture, Republic of South Africa* **191**, 58-62.

Flint, M.L.; Van den Bosch, R. (1981) *Introduction to integrated pest management.* Plenum Press, New York.

Steel, R.G.D.; Torrie, J.H. (1960) *Principles and procedures of statistics, a biometrical approach.* McGraw-Hill, New York.

Van der Westhuizen, M.C. (1992) Insekplaagbeheer. In *Plantbeskermingskursus: Glenkovs Beskerm en Bewaar.* Eds. M.C. van der Westhuizen, AVCASA, N.C.J. Basson, J. de Jager, D. du Toit & E. Wolmarans, University of the O.F.S. Bloemfontein, pp 70-126.

Zadoks, J.C.; Chang, T.T.; Konzak, C.F. (1974) A decimal code for the growth stages of cereals. *Weed Research* **14**, 415-421.

Zar, J.H. (1974) Biostatistical analysis. In *Biological Sciences Series.* Eds W.D. McElroy & C.P. Swanson. Prentice-Hall, Englewood Cliffs, N.J, pp 228-235.

IMPACT OF SEED-COATING POLYMERS ON MAIZE SEED DECAY BY SOILBORNE PYTHIUM SPECIES

D. C. McGEE, B. ARIAS-RIVAS, J. S. BURRIS

Seed Science Center and Departments of Plant Pathology and Agronomy, Iowa State University, Ames, IA 50011, U.S.A.

ABSTRACT

Polymeric seed coatings were investigated as alternatives to fungicide seed treatments of maize. A field study was carried out in which three polymers either alone or in combination with the standard captan seed treatment of maize were examined for plant emergence and infection of nongerminated seeds by soilborne *Pythium* species. Polymers in combination with captan were as effective as captan alone in ensuring an adequate stand of maize seedlings and in reducing incidence of *Pythium* spp. seed infection. There was little evidence that seed treatment with polymers alone could provide protection against infection and improve plant emergence.

INTRODUCTION

Pythium species are soil-inhabiting fungi that cause seed rot and damping-off in a wide range of plants. Maize seeds are particularly vulnerable to attack when subjected to cold and wet soil conditions after planting (Johann, H. *et al*, 1928 and Rao, B. *et al*, 1978). Effective control can be achieved by seed treatment with the fungicide captan. However, increasing concerns about human health risks and environmental contamination have stimulated research for alternatives to fungicide and reduction in fungicide dosages.

There has been considerable interest in the use of degradable polymeric seed coatings as carriers for chemical and biological control agents in recent years. The potential of polymers to improve retention of the active ingredient on seeds could lead to lower dosages of fungicides and improved efficacy. Polymers also can regulate the rate of water imbibition by seeds which could allow seeds to escape infection by soilborne pathogens. It has been shown that polymer coatings can reduce invasion of maize and soybean *Aspergillus* and *Penicillium* spp. in seeds stored at high relative humidity by reducing the rate of moisture uptake (McGee, D. C. *et al*, 1988).

The present study investigated the potential of seed treatment of maize with polymers, either alone or in combination with captan, to ensure adequate plant stands and reduce seed decay by soilborne *Pythium* spp. under field conditions.

MATERIALS AND METHODS

Seed treatment

Polymers examined included Sacrust (30% liquid suspension) manufactured by Sarea, Luiz, Austria; Chitosan (2.5% liquid suspension manufactured by Nova Chem Limited, Halifax, Nova Scotia, Canada; and Certop (30% liquid suspension) manufactured by Cel-Pril, Manteca, California, U.S.A. Two hybrid maize seed lots were treated with these polymers at the rate of 125 ml/kg seed either alone or in combination with captan (Captan 400-D)at 500 ppm a.i. Treatments were applied in fluidized bed coater as described previously(Burris, J. S. *et al*, 1994). Separate lots of each hybrid also were treated with captan (Captan 30DD) at 500 ppm a.i. and metalaxyl (Apron-FL) at 318 ppm a.i. in a Batch Laboratory Treater (Gustafson Inc., Dallas, Texas, U.S.A.).

Field experiment

Seeds were planted in the field on April 30, 1993 near Ames, Iowa. Individual plots comprised four rows, 5 m long and 75 cm wide, with 100 seeds planted per row at a depth of 6 cm. A split-split-plot experimental design was used with three replications. Main plots were the high and low vigor seed lots, sub-plots were the polymer treatments alone or in combination with captan, captan or metalaxyl alone, and untreated seeds. Sub-sub-plots were evaluation dates for plant emergence and seed colonization by *Pythium* spp. Seedling emergence in the middle two rows was counted at 12, 16, 20 and 25 days after planting. All nongerminated seeds from a 1 m row section in one of the outside rows of each plot were removed at 4, 8 and 15 days after planting. Ten seeds from each plot were randomly selected and washed with tap water for 10 min. Seed coats and embryos were dissected aseptically from each seed and plated separately on a medium selective for *Pythium* spp. (Schmitthenner, 1980). Plates were incubated in the dark at 25 C for 4 days and number of colonies of *Pythium* spp. growing from seed coats and embryos were recorded.

RESULTS

Field emergence

Relative differences in seedling emergence between treatments were similar at all times of evaluation, therefore only data for the final count (25 days after planting) are presented (Table 1). Main effects in the statistical analysis showed that emergence of high vigor seeds at 25 days after planting was significantly greater than that for low vigor seeds. The emergence of seeds treated with Chitosan alone did not differ significantly from that for seeds treated with Chitosan combined with captan for the high vigor seed lot, but it was significantly lower for the low vigor lot (Table 1). For all others polymers, seedling emergence was significantly greater when they were applied with captan than when applied alone for both seed lots. Seedling emergence for all captan/polymer combinations was not significantly different from that for seed treated with captan alone or metalaxyl in both seed lots. Polymers applied alone did not significantly improve seedling emergence over that for untreated high vigor seed lot, but Sacrust and Chitosan showed a small but significant increase over untreated low vigor seeds.

TABLE 1. Effect of polymer and fungicide seed coatings on seedling emergence and *Pythium* spp. infection of maize seeds.

Seed treatment	Seedling emergence (%)	*Pythium* spp. infection (%) of nonemerged seeds	
		Seed Coat	Embryo
HIGH VIGOR SEED			
Sacrust	65.0	53.3	26.7
Sacrust + captan	93.3	3.3	16.7
Chitosan	86.2	52.3	21.0
Chitosan + captan	86.7	10.0	6.7
Certop	77.3	3.3	10.0
Certop + captan	92.5	7.0	3.3
Captan	92.7	11.0	17.0
Metalaxyl	88.2	27.0	10.0
Untreated	80.8	20.0	20.0
LSD (P=0.05)	7.2	17.5	19.0
LOW VIGOR SEED			
Sacrust	58.5	31.0	47.7
Sacrust + captan	69.3	3.3	3.3
Chitosan	49.8	48.7	62.3
Chitosan + captan	68.3	6.7	6.7
Certop	34.7	36.7	43.3
Certop + captan	81.3	3.3	6.7
Captan	77.5	3.3	31.0
Metalaxyl	64.0	0.0	21.0
Untreated	41.3	21.0	47.0
LSD (P=0.05)	7.2	25.6	20.0

Seed infection by *Pythium* spp.

Relative differences in *Pythium* infection of nongerminated seeds was similar at each sampling time, therefore only data for 8 days after planting are presented (Table 1). No damped-off seedlings were detected in the experiment. Seed coat and embryo infections were consistently higher for seeds treated with polymer alone than with polymer combined with captan or captan alone for low vigor seeds. The same trend occurred for high vigor seed, but differences were significant only for Sacrust. No differences in infection of seed coats occurred for seed coated with polymer combined with captan and captan alone. Embryo infection of low vigor seeds however, was significantly less for seeds treated with polymer combined with captan compared to captan alone. No significant differences were evident between *Pythium* infection for seeds treated with the two fungicides alone.

DISCUSSION

This study has shown that treatment of maize seeds with degradable polymers in combination with captan is as effective as captan alone in ensuring adequate seedling stands and in reducing infection of seeds by *Pythium* spp. under field conditions. These effects were particularly evident for the low vigor seed lot. The test was conducted under conditions favorable for root pathogens such as *Pythium* spp. in that the soil was much wetter than normal in the four week period after planting. It is of interest that extensive *Pythium* infection occurred on nongerminated seeds, regardless of the original vigor of the lot. There was little evidence that seed treatment with polymers alone could provide protection against infection by *Pythium* spp. and improve plant emergence. Emergence was similar for seeds treated with Chitosan alone and Chitosan combined with captan, but this was seen only for the high vigor seed lot and was not reflected in *Pythium* control.

This is one of the few studies of maize seed treatments, in which pathogen control was related to seedling emergence under field conditions. It provides a starting point for further research on the mechanism of control of soilborne pathogens of maize with polymer seed treatments. While the data did not show promise for the use of polymers alone in control of seedling disease, findings were only for one growing season and this approach should not be abandoned. The fact that the addition of polymers to captan seed treatment did not compromise the efficacy of captan is an encouraging finding for further research on the use of polymers as a means of reducing the captan dosage.

ACKNOWLEDGEMENTS

Journal Paper J-15572 of the Iowa Agriculture and Home Economics Experiment Station. Project 3099.

REFERENCES

Burris, J. S.; Prijic, L. M.; Chen, Y. (1994) A small scale laboratory fluidized bed seed-coating apparatus. These Proceedings.

Johann, H.; Holbert, J. R.; Dickson, J. G. (1928) A Pythium seedling blight and root rot of dent corn. *J. Agric. Res.* **37**,443-464.

McGee, D. C.; Henning, A.; Burris, J. S. (1988) Seed encapsulation methods for control of storage fungi. In: *Application to Seeds and Soil*, T. J. Martin (Ed.) BCPC Monograph No. 39, Thornton Heath: BCPC Publications, pp.257-264.

Rao, B.; Schmitthenner, A. F.; Caldwell, R.; Ellett, C. W. (1978) Prevalence and virulence of *Pythium* species associated with root rot of corn in poorly drained soil. *Phytopathology* **68**:1557-1563.

Schmitthenner, A. F. (1980) *Pythium* species: isolation, biology and identification. In: *Advances in Turfgrass Pathology*, P. O. Larsen and B. G. Joyner (Eds.) Duluth, Minnesota, Harcourt Brace Jovanovich, pp. 33-36.

THE ROLE OF CARBOXIN + THIRAM FS IN POST-MERCURY SEED TREATMENT FUNGICIDE STRATEGIES IN CENTRAL EUROPE

D.JACKSON, R.MARSHALL, M.J.TOMKINS
Uniroyal Chemical Ltd, Kennet House, 4 Langley Quay,
Slough, Berkshire SL3 6EH

O.NOVY
Uniroyal Chemical, Jankovcova 18, 17037 Praha 7, Czeck

Z.PAPP
Uniroyal Chemical Office, Timar Utca 20, H-1034, Budapest

J.ROMANIUK
Uniroyal Chemical, PO Box 301, Warsaw, Poland

ABSTRACT

Organomercurial seed treatments were widely used in Central Europe particularly on small-grain cereals; all seed treatment uses have now been terminated, except in Romania.

Specific and broad-spectrum replacement fungicides have been developed and the range of products now available is reviewed.

Carboxin + thiram FS has been widely tested in Central Europe and demonstrates broad-spectrum disease control coupled with multi-crop use and seed safety. It is easy to apply and has a direct stimulatory effect on emergence and growth of seedlings. Carboxin + thiram FS is registered internationally which will help movement of treated seed in and out of Central Europe.

INTRODUCTION

Organomercury seed treatments became widely accepted in Central Europe as much as in West Europe because of their economic and effective control of important seed-borne diseases. In spite of the effectiveness of mercury these diseases were not eradicated altogether. Certain weaknesses in performance and gaps in the spectrum of mercury were identified in Central Europe as elsewhere, and development started with alternative chemicals. Replacement products were available by the 1970's, and the gradual prohibition of mercury seed treatments in Central Europe started.

The importance of seed-borne diseases and the need to use effective seed treatment chemicals continues to be recognised in Central Europe for the same reasons as in the UK (Rennie, 1993). Seed treatment is still the only means of controlling certain diseases of cereals and other crops; it is a cost-effective measure and is a key element of low-input systems and integrated developments typical of Central Europe. Improvement of seed health through use of seed treatments is also an important part of the development of seed production business there.

This paper will review the modern seed treatments available in Central Europe, and will present developments with carboxin + thiram formulations mainly on cereals and from Poland, Hungary, Czech Republic, Slovak Republic and Romania.

ORGANOMERCURY STATUS IN EUROPE

Organomercury seed treatments are being prohibited in Central Europe because of problems of acute toxicity and effect on wildlife. Poland (1977) and Hungary (1978) were the first to prohibit mercury use as seed treatments, with the Czech and Slovak Republics following much later (1991) and Bulgaria and Yugoslavia in 1992. There are derrogations in certain Yugoslavian republics, and use is still permitted in Romania. Some countries, such as Hungary, are particularly concerned about safety to birds. Environmental pollution concerns, particularly at sites of manufacture have been a strong stimulus for replacing mercury in recent years.

NON-MERCURIAL SEED TREATMENTS

A wide range of modern fungicides is now available for use as seed treatments, (Noon & Jackson, 1992) and can as replace mercury. The replacement in Central Europe has been made partly with some of these modern, often systemic, molecules and partly with traditional commodity contact fungicides.

The newer products imported from the West, tend to be used for certified seed production and in areas of highest disease risk. The largest volume seed treatments are still the locally manufactured contact fungicides.

The most commonly used mercury alternatives are listed below (Table 1).

Typically only a few products are used on seed in most countries because mercury replacement is relatively recent. Hungary has the widest range of replacement products available.

Most seed treatment fungicide use is targeted against seed-borne pathogens, with relatively little use made of systemic seed treatments for control of foliar diseases.

Application and formulation improvements are becoming more important as more modern chemicals become available. Until relatively recently only powder formulations were used, applied either dry or as slurries. Slurry application is still important but more countries are introducing suspension concentrate (FS, flowable) products. This development has paralleled the use of more sophisticated application equipment e.g. Czech and Slovak Republics, Hungary.

TABLE 1. Seed treatment fungicides registered in Central Europe.

Chemical Group	Chemical
Dithiocarbamates	Thiram, maneb/mancozeb/zineb
Copper salts	Copper hydroxyquinolate
Benzimidazoles	Carbendazim, fuberidazole, thiophanate methyl
Carboxamides	Carboxin
Guanidines	Guazatine
Ergosterol Biosynthesis Inhibitors	Flutriafol, triadimenol, tebuconazole, diniconazole, imazalil, bitertanol
Phthalimides	Captan

CARBOXIN SEED TREATMENTS

Carboxin was first introduced by Uniroyal Chemical as a systemic seed treatment for control of Basidiomycete pathogens such as *Ustilago* spp and *Tilletia* spp (Kulka & Schmeling,1987).It is now recognised that carboxin is also active against other pathogens such as *Pyrenophora* spp and *Fusarium* spp, although coformulation with partner fungicides is needed for full control. Carboxin + thiram is one of the most common mixtures used worldwide.

Carboxin + thiram is now widely developed and registered as a suspension concentrate formulation containing 200g/l each of carboxin and thiram, trade name Vitavax 200FF. This formulation has improved characteristics of uniformity of application and adhesion, and can be applied undiluted or diluted with water according to local conditions.

Results of efficacy and crop stimulation trials are presented below.

RESULTS

Efficacy

Results of small-plot trials are presented for the major seed-borne diseases. Trials were carried out with naturally infected seed treated in the laboratory, using commercial formulations diluted with water to give a total application volume of 1 l/100kg seed.

Bunt (*Tilletia caries*) on wheat

A summary of 6 trials in 4 countries against *T.caries* is given in Table 2. The trials were all done with artificially inoculated seed (2g spores/kg) and produced a wide range of symptom levels. Carboxin + thiram FS performed as well as the reference products, with all treatments giving good control.

TABLE 2. Efficacy of carboxin + thiram FS against *T.caries*

Treatment	Rate	Percent control					
	g ai/100kg	Slovakia		Hungary	Czech	Romania 1992	
		1991	1992	1991	1992	1	2
Untreated (% infected ears)	-	(3.5)	(9.3)	(21.4)	(4.2)	(56)	(9.8)
Carboxin + thiram FS	40 + 40	100	99.9	100	-	-	-
	60 + 60	100	100	-	100	92.8	93.9
Tebuconazole	3	-	100	-	-	-	-
Organomercury	2	-	-	-	-	64.3	69.4

Fusarium seedling blight

Seed and seedling blight caused by *Fusarium* spp is a major problem in Central Europe, and data from a trial in Hungary on wheat cv Mv-14 are shown in Table 3.

Carboxin + thiram FS increased germination levels and emerged plants in the field, giving a final increase in yield. *Fusarium* levels on the seedlings were decreased by all products.

Leaf stripe (*Pyrenophora graminea*)

Barley leaf stripe is a severe disease in some areas of Central Europe, and seed treatment must be used to ensure seed certification and protect yields.

Results of five trials are summarised in Table 4; carboxin + thiram FS gave greater than 90% control at 60 + 60 g ai/100kg in 4 trials, even under conditions of high infection pressure.

Seed safety and crop stimulation

Carboxin + thiram FS seed treatment is very selective for most types of seed under standard germination conditions, even at high doses and after storage of treated seed.

A stimulatory effect on seedlings can often be seen, and Table 5 shows the results of carboxin + thiram seed treatment on the field emergence of winter wheat, with a strong positive effect in 2 out of 3 cultivars tested.

TABLE 3. Control of *Fusarium* seedling blight on wheat.

Treatment	Rate g ai/100kg	Percent germination	% Infected Seedlings	Plants/ m/row	Yield t/ha
Untreated control	-	90.0	18	64	4.9
Carboxin + thiram FS	50 + 50	97.5	0	77	5.2
Guazatine	70	97.5	2.5	72	5.2
Carbendazim + Cu oxyquinolate	30 + 30	97.0	0.5	80	5.1

TABLE 4. Control of leaf stripe on barley

Treatment	Rate g ai/100kg	Percent control Slovakia (1)	Percent control Slovakia (2)	Romania (1)	Romania (2)	Romania (3)
Untreated control (percent infection)	-	(3.8)	(9.5)	(22.0)	(30.3)	(9.7)
Carboxin + thiram FS	40 + 40	93.4	74.1	-	-	-
Carboxin + thiram FS	60 + 60	100	86.7	93.2	92.1	94.8

TABLE 5. Field emergence of winter wheat, Poland, 1991.

Treatment	Rate	Relative emergence Cultivar A (9 trials)	Cultivar B (9 trials)	Cultivar C (9 trials)
Untreated control	-	100	100	100
Carboxin + thiram FS	60 + 60	113	110	101
Reference Products	(various)	109	-	94

One example of the effect of carboxin + thiram on early growth of seedlings is shown in Table 6, where treated seedlings produced larger root masses compared to non-treated seeds.

TABLE 6. Root growth of 3 crops treated with carboxin + thiram (Hungary, 1991, pot trial).

Treatment	Rate	Root Growth Wheat length	 Wheat wt	 Maize length	 Maize wt	 Sunflower length	 Sunflower wt
Untreated control	-	100	100	100	100	100	100
Carboxin + thiram FS	60 + 60	140	145	111	143	121	114

The subsequent benefits of these early growth effects have been followed to yield in many large-scale trials throughout Europe; carboxin + thiram FS - treated seed consistently produces more vigorous crops than untreated seed, particularly under adverse growing conditions or with late sowing. For example 135 yield trials carried out in 1992 show that carboxin + thiram gave an average of 11.6% higher yields across a wide range of cereal types and growing conditions. It was noticed that the largest differences were produced on lower-input, low yielding crops.

DISCUSSION AND CONCLUSIONS

Seed treatments continue to be an important part of crop protection after mercury withdrawals in Central Europe. A range of alternative products is registered and the choice will depend on cost-effectiveness and availability. Imported systemic products are gradually replacing local contact chemicals, and improved application techniques are being used with more sophisticated formulations.

A new formulation of carboxin, carboxin + thiram FS, gives broad-spectrum disease control of small-grain cereals, is easy to apply and is very selective. Positive growth benefits have been demonstrated with the product, even in the absence of significant disease infections. This growth stimulation is thought to originate from the PGR effects of carboxin (Schmeling & Clark 1970). Coleoptile length in cereals has also been shown to be increased with carboxin treatment (Gustafson, unpub.). Faster and stronger early seedling growth is important under adverse conditions eg. late drilling, deep sowing. The combination of seed safety, stimulated growth and disease control gives an increase in crop uniformity and yield.

Carboxin + thiram FS is now registered in all countries of Central Europe on cereals and a range of other crops including maize, peas, soyabean & vegetables at a rate of 2.5 to 3l/T.

REFERENCES

Kulka, M.; Schmeling, von B. (1987) Carboxin fungicides and related compounds. *In:Modern Selective Fungicides, J.Lyr (Ed.)*, VEB Gustav Fisher Verlag Jena, pp. 119-132.

Noon, R.A.; Jackson, D. (1992) Alternatives to mercury for control of cereal seed-borne diseases. *British Crop Protection Conference - Pests and Diseases 1992*, **3**, 1127-1136.

Rennie, W.J. (1993) The need for cereal seed treatment in the UK in the post-mercury era. *Pesticide Outlook*, **4** (1), 19-14.

Schmeling, B.von; Clark, M.L. (1970) Oxathiin induced plant growth stimulation. *VII International Congress of Plant Protection*, Section B314.

THE PHENYLPYRROLES: THE HISTORY OF THEIR DEVELOPMENT AT CIBA

N.J. LEADBITTER, R. NYFELER, H. ELMSHEUSER

Ciba-Geigy Limited, Basle, Switzerland

ABSTRACT

Fenpiclonil and fludioxonil are two fungicides from phenylpyrrole chemistry that have been developed by Ciba. They are both highly active as seed treatments in a wide range of crops.

Their development at Ciba was based on the lead structure pyrrolnitrin, a natural antimycotic. Using the TOSMIC route many 4-phenylpyrroles were synthesised and tested for efficacy. The two most active compounds, fenpiclonil and fludioxonil were chosen for further development. Biological field testing, toxicological investigations, mode of action studies, formulation development, registration and marketing were all needed to bring these compounds to the market.

INTRODUCTION

In modern pest management there is a constant need for new seed treatment products. Replacements are clearly required for products that have been taken off the market or banned e.g. mercury. The usefulness of other active ingredients e.g. carbendazim has been reduced due to the development of resistance following their widespread use over a number of years. New products are needed which can control resistant pathogens. Furthermore the needs of the user and consumer can change and it is important that new products are developed which fulfil them.

The need for new products is therefore clear and ideally a modern seed treatment should combine excellent crop tolerance with a spectrum of activity that enables it to replace the older products, has low use rates and a favourable toxicology and ecotoxicology. Also new products should preferably belong to classes of chemicals which do not show cross resistance to currently used seed treatments.

It was in this environment that Ciba began the development which led to the introduction of two new active ingredients for seed treatment, fenpiclonil and fludioxonil. This paper describes the background to that development.

DISCOVERY

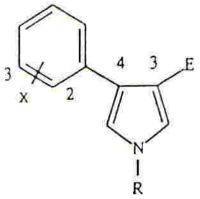
In the the mid 1960s pyrrolnitrin, a secondary metabolite produced by different *Pseudomonas* spp and various species from the myxobacteriales, was first isolated from *Pseudomonas pyrocinia* (Arima *et al*, 1965). Due to its interesting antifungal activity, pyrrolnitrin was developed as an antimycotic for topical application in human medicine and also served as a lead structure for pharmaceutical research (Suminori *et al*, 1969). The first use of a pyrrolnitrin analogue in plant protection was described in 1969 in a Japanese patent application (Nakanishi *et al*, 1975) and in greenhouse tests at Ciba in the late 1970s pyrrolnitrin showed interesting activity against a range of phytopathogenic fungi such as *Botrytis cinerea* and *Pyricularia oryzae*. However, pyrrolnitrin has a major drawback in that it is highly light labile (Ueda *et al*, 1982) making it unsuitable for use as a commercial fungicide.

Synthetic chemists at Ciba therefore set about the task of investigating the structural/activity relationships in attempt to identify structures that were potential commercial compounds.

Despite its apparently simple structure pyrrolnitrin proved to be a challenge for the synthetic chemists. The most useful synthesis described at that time was considered to be that described by Gosteli (1972) a method which required six steps and gave a yield of only 20%. This approach was not considered attractive for the synthesis of a large number of analogues of pyrrolnitrin. However, a rather simple process to prepare novel pyrroles had been described by van Leusen *et al* (1972) using TOSMIC (p-toluenesulfonyl methylisocyanide) as a key reagent.

Using the TOSMIC route different types of 4-phenylpyrroles were synthesized and their fungicidal activity tested. The influence of the substituents E, X and R as well as the position of X in the structure in figure 1 was tested. Results indicated that among the compounds prepared the 3-cyanopyrroles (E = -CN) had the highest activity. Furthermore this work also showed that the substituent X should be an electron withdrawing group (in these tests -Cl, -Br, CF_3, $-0CF_20-$) and that they should be substituted at the -2 and - 3 position on the phenyl ring. Highly active compounds were found with the 2,3-dichloro- and 2.3($-0CF_20-$) substituted phenyl derivatives. For the N-substituted derivatives of the 3-cyano-4-phenylpyrroles it was also observed that only those with R substituents that easily hydrolysed back to the parent compound showed high fungicidal activity.

Figure 1: General formula for the 4-phenylpyrroles



Therefore, at Ciba it was found that 3-cyano-4-phenylpyrroles with substituents in either 2- or 3- position and preferably in the 2- and 3- position of the phenyl ring proved to be most interesting. Of these compounds fenpiclonil (synthesized 1982) and fludioxonil (synthesized 1984) (Fig 2) were selected for development. Further details of this work can be found in Nyfeler & Ackermann (1992).

Figure 2: Formulae of fenpiclonil and fludioxonil

fenpiclonil fludioxonil

BIOLOGICAL DEVELOPMENT

Following synthesis, both compounds were rigorously screened and shown to have a similar spectrum of activity against a range of *Deuteromycete* , *Ascomycete* and *Basidiomycete* fungi when applied as either a foliar spray or as a seed treatment (Nevill *et al*, 1988; Gehmann *et al*, 1990). They were also very well tolerated by a wide range of crops.

TABLE 1. Activity of fenpiclonil (20g AI/100kg seed) and fludioxonil (5g AI/100kg seed) against seed-borne diseases of wheat and barley.

Pathogen(s)	Disease	Activity compared with mercury
Wheat		
Gerlachia nivalis	Snow mould	greater than
Fusarium spp	*Fusarium* seedling blight	greater than
Tilletia caries	Common bunt	equal to
Septoria nodorum	*Septoria* seedling blight	equal to
Urocystis agropyri	Flag smut	no data
Helminthosporium sativum	*Helminthosporium* seedling blight	greater than
Barley		
Gerlachia nivalis	Snow mould	greater than
Fusarium spp	*Fusarium* seedling blight	greater than
Helminthosporium sativum	*Helminthosporium*	greater than
Helminthosporium gramineum	Leaf stripe	equal to*
Ustilago hordei	Covered smut	no data

* fenpiclonil and fludioxonil will control mercury resistant isolates

Field trials using the phenylpyrroles as seed treatments have been carried out since the mid 1980s. So far more than 1000 trials have been initiated on an ever widening range of crops. Initial data identified that both fenpiclonil and fludioxonil were active against a broad spectrum of diseases of wheat and barley with a spectrum broader than that of mercury (Table 1, for further details see Leadbeater *et al*, 1991; Koch & Leadbeater, 1992). Investigations of dose response on cereals showed that fludioxonil is inherently more active than fenpiclonil. The level of control given by 5g ai/100kg fludioxonil being at least equivalent to that given by 20g ai/100kg fenpiclonil.

Further work showed that on other pathosystems fenpiclonil and fludioxonil, also have good activity as seed treatments. On potatoes both compounds control a wide range of seed-borne diseases (Table 2) and on peas both show activity against *Ascochyta pisi* and *A. pinodes*. Fludioxonil also has activity against seed-borne pathogens on rice, maize, cotton and a number of other crops (Leadbeater *et al*, 1991; Koch & Leadbeater, 1992).

TABLE 2. Activity of fenpiclonil (50g AI/tonne tubers) and fludioxonil (20-25g AI/tonne tubers) when used as a pre-plant application to potatoes.

Pathogen*	Disease	Activity
Rhizoctonia solani	Stem canker Black scurf	= pencycuron
Heminthosporium solani	Silver scurf	= imazalil & carbendazim**
Fusarium spp	Dry rot	= thiabendazole**
Polyscutalum pustulans	Skin spot	= thiabendazole

* seed-borne infections ** will control isolates resistant to carbendazim

In situations where resistance has developed to currently used products it is important that new products have activity against isolates of pathogens insensitive to those products. In many cases the phenylpyrroles have activity against pathogens where carbendazim has commonly been used. At an early stage therefore the phenylpyrroles were tested against isolates of pathogens that had developed resistance to carbendazim. In all cases the phenyl pyrroles have been shown to be active against these resistant isolates both *in vtro* and *in vivo*. The pathogens tested so far are *Fusarium nivale* (wheat), *Ascochyta pisi* and *Ascochyta pinodes* (peas), *Gibberella fujikuroi* (rice), *Helminthosporium solani* (potatoes), *Fusarium solani* (potatoes).

MODE OF ACTION

Although mode of action studies were initiated early in the development of the phenypyrroles the mode of action is still not well understood. Studies with fenpiclonil show that there is no inhibition of respiration, chitin-, DNA-, or RNA-synthesis and it does not interfere with ergosterol or lipid biosynthesis. However, it caused a fast inhibition of mycelial growth as well as an instantaneous reduction in amino-acid uptake (Jespers & Davidse, 1990). Leroux (1991) suggested that fenpiclonil has a similar mode of action to dicarboxamides like iprodione and aromatic derivatives such as toclofos-methyl but the mode of action of these compounds is also not clearly understood and the suggested similarity has not been confirmed. Further work by Jespers *et al* (1993) has indicated that cell membrane transport processes are affected and they concluded that to their knowledge such a mechanism had not been described before for any fungicide. Further work is in progress to define more precisely the mode of action of these compounds.

TOXICOLOGY AND ECOTOXICOLOGY

Toxicological and ecotoxicological studies were started early in the development of the phenylpyrroles. Data generated from many studies has shown that the phenylpyrroles have good applicator, consumer and environmental safety.

FORMULATIONS

The development of a high quality formulation for new active ingredients is a vital part of the development process. In many cases the formulation can affect the efficacy of the chemical and also its safety to the crop. With the phenylpyrroles it was decided that water based flowable formulations (FS) should be developed to reduce the likelihood of damage to the seed which may be caused by solvent based formulations and also to provide products which are as safe as possible for the user.

Another issue regarding formulations is their ease of use. To this end FS formulations of both fenpiclonil and fludioxonil have been designed as "ready to use" formulations. For example fenpiclonil for use on wheat and barley has been formulated as a FS050 with an application rate of 400ml/100kg giving good application properties without the need for dilution. For further details of formulation development see Frank (1994).

MARKETS AND REGISTRATION

Since both compounds were shown to have broad spectrum activity against diseases on wheat and barley the first major markets were identified as the cereal growing areas of Northern Europe. Fenpiclonil was first introduced in Switzerland in 1988 and by 1990 had achieved 50% of the wheat seed treatment market. It was subsequently registered in other European countries and gained registration in the United Kingdom during 1993. The first registration for fludioxonil on cereals was achieved in France in 1993 and registrations in other European countries are expected in the near future.

As the spectrum of activity on other crops was elucidated so further markets were identified. Fenpiclonil is already registered for use on potatoes in Belgium and has been submitted for registration in a number of other countries. Although fenpiclonil will only be developed for European markets, fludioxonil will be marketed worldwide. As well as for control of diseases on cereals and potatoes it will also be used on peas, maize, cotton and rice, alone and in mixture with other active ingredients.

ACKNOWLEDGEMENTS

The authors would like to thank all those who contributed to the successful development of fenpiclonil and fludioxonil.

REFERENCES

Arima, K.; Imanaka H.; Kousaka, M.; Fukuda, A; Tamura, G. (1965) Studies on pyrrolnitrin, a new antibiotic. 1: Isolation and properties of pyrrolnitrin. *Journal of Antibiotics*, 18, 211-219.

Frank, L (1994) The life cycle of a seed treatment formulation development. *These proceedings.*

Gehmann, K.; Nyfeler, A.J.; Leadbeater, A.J.; Nevill, D.; Sozzi, D. (1990) CGA173506: A new phenylpyrrole fungicide for broad spectrum disease control. *Brighton Crop Protection Conference - Pests and Diseases 1990*, 1, 399-406.

Gosteli, J. (1972) New synthesis of the anitbiotic pyrrolnitrin. *Helv. Chim. Acta*, 55, 451-460

Jespers, A. B. K.; Davidse, L. C. (1990) *Abstract of the 4th International Mycological Congress*, Regensburg, 286.

Jespers, A. B. K.; Davidse, L. C.; de Waard, M. A. (1993) Biochemical effects of the phenylpyrrole fungicide fenpiclonil in *Fusarium sulphureum* (Schlect). *Pesticide Biochemistry and Physiology*, 45, 116-129.

Koch, E; Leadbeater, A.J. (1992) Phenylpyrroles - a new class of fungicides for seed treatment *Brighton Crop Protection Conference - Pests and Diseases 1992*, 3, 1137-1146.

Leadbeater, A.J.; Nevill. D; Steck. B; Nordmyer, D. (1990) CGA173506: A novel fungicide for seed treatment. *Brighton Crop Protection Conference - Pests and Diseases 1990*, 2, 825-830.

Leroux, P; Lanen, C; Fritz, R. (1992) Similarities in the antifungal activities of fenpiclonil, iprodione and toclofos-methyl against *Botrytis cinerea* and *Fusarium nivale*. *Pesticide Science*, 36, 255-261.

Nakanishi, K; Shimizu, K; Ouo, K.; Ueda, K. (1975) Japanese Patent 50,002,011 (Appl. 19.3.1969), *Chemical Abstracts*, 83, 38796.

Nevill, D.; Nyfeler, R; Sozzi, D; (1988) CGA142705: A novel fungicide for seed treatment *Brighton Crop Protection Conference 1988*, 1, 65-72.

Nyfeler, R.; Ackermann, P. (1992) Phenylpyrroles, a new class of agricultural fungicides related to the natural antibiotic pyrrolnitrin. In *Synthesis and Chemistry ofAgro-chemicals*, D.R. Baker, J.G. Fenyes & J.J. Steffens(Eds), American Chemical Society pp395-404.

Suminori, U.; Kazuo, K.; Tanaka, K.; Nakamura, H. (1969) *Chem. Phar. Bulletin (Tokyo)*, 17, 559-566.

Ueda, A; Nagasaki, H.; Takakura, Y.; Nishikawa, H; Nakada, A. (1982) *European Patent Application* 92, 890.

van Leusen, A.M.; Siderius, H.; Hoogenboom, B.E.; van Leusen, D. (1972) Chemistry of sulfonylmethyl isocyanides. 6. New and simple synthesis of the pyrrole ring system from Michael acceptors and (p-tolylsulfonyl) methyl isocyanide. *Tetrahed Letters*, 5337-5340.

DRESSING ZONE FORMATION, UPTAKE, TRANSLOCATION AND ACTION OF [^{14}C]IMIDACLOPRID FOR WINTER WHEAT AFTER SEED TREATMENT AND UNDER THE INFLUENCE OF VARIOUS SOIL MOISTURE LEVELS

U. STEIN-DÖNECKE, F. FÜHR, J. WIENEKE

Institute of Radioagronomy, Research Centre Jülich, 52425 Jülich, Germany

ABSTRACT

After seed treatment with [^{14}C]imidacloprid, winter wheat was cultivated at three different soil moisture levels. Higher soil moisture contents promoted the removal of ^{14}C activity from the caryopsis and distribution in the soil, and thus reduced both the potential availability of the active ingredient in the soil and also the incorporation of ^{14}C into the plant. Between 7 and 22 % of the ^{14}C activity applied per caryopsis was incorporated into the wheat shoot by the end of shooting. Up to this point in time, the concentration of active ingredient equivalents in the shoot decreased greatly as a consequence of an increasing difference between the subsequent delivery of ^{14}C and increase in new growth. A concentration gradient decreasing from the oldest to the youngest leaf for ^{14}C and imidacloprid points to an apoplastic translocation. Lower ^{14}C incorporation into the plant and lower active ingredient fractions of the ^{14}C activity in the leaf at higher soil moisture levels resulted in reduced concentrations of extractable imidacloprid in the leaf. Nevertheless, even 195 days after sowing a high toxicity to aphids was still maintained at concentrations between 12 and 110 µg of imidacloprid/kg fresh weight in the youngest leaves.

INTRODUCTION

The development of a new active ingredient, imidacloprid, means that a systemic insecticide for the seed treatment of cereals is available, with which a long-term control of aphids, and also a reduction in infection with the Barley Yellow Dwarf Virus (BYDV) can be achieved (Schmeer *et al.*, 1990). The aphid infestation responsible for transmitting the BYDV, and thus the associated reductions in yield, sometimes only takes place in spring in the case of winter wheat (Huth, 1990). This means that after seed treatment with imidacloprid sufficient active ingredient must be available in the plant or supplied from the soil even at this late point to control the aphids and prevent virus transmission. In order to quantify these factors, dressing zone formation, i.e. active ingredient distribution and availability in the soil, as well as uptake, translocation and action in the plant were recorded until the spring after seed treatment of winter wheat with ^{14}C-labelled imidacloprid in an extensive study. Since it is known from studies on the uptake of triadimenol after the dressing of winter barley (Schneider, 1988) that soil moisture can have a great influence on dressing zone formation and active ingredient uptake, the present studies were carried out with three different soil moisture contents. Some of the results obtained are presented below.

MATERIALS AND METHODS

Seed Treatment

Winter wheat (Kanzler) was treated on a laboratory scale with [pyridinyl-^{14}C-methyl]imidacloprid as a 70WS formulation, applied at 100 g a.i./100 kg seed. The analysis of 50 of a total of 850 treated caryopsis resulted in a mean ^{14}C activity of 78 kBq/caryopsis; a quantity of 50.2 µg imidacloprid/caryopsis was thus calculated at a specific ^{14}C activity of the active ingredient applied of 1.554 MBq/mg.

Experimental Setup, Sowing, Sampling and Preparation

The winter wheat was sown on 20.11.1990 in containers 1 m^2 in size filled with the topsoil of a degraded loess soil (Parabraunerde); seeds were sown every 2 cm in rows 12 cm apart at a depth of 2.5 cm. The plants were cultivated in the open air at three different soil moisture contents, amounting to 30, 40 and 50 % of the maximum water holding capacity (WHC_{max}) throughout the entire experiment.

The studies of dressing zone formation and ^{14}C uptake were carried out for all three soil moisture levels at three stages of development (start of tillering (21), start and end of the shooting phase (31 and 45); 111, 153 and 195 days after sowing respectively) on the basis of 10 plants and soil samples in each case. A further 10 plants served to determine the active ingredient contents and concentrations of selected leaves. The uptake and distribution of the ^{14}C activity in the plant was determined on 7 additional dates, from the 1-leafstage (11), 51 days after sowing, untill full ripeness (91), 260 days after sowing, but only for 40 % WHC_{max} .

Steel frames (15 cm deep, 2 x 12 cm) were used to remove the plants with their associated dressing zone covering the soil up to the adjacent dressing zones and permitting a subdivision into the region close to the grain (5 cm deep, 2 x 5 cm) and the remaining dressing zone. The plants were divided into caryopsis, root and shoot, and some of them also into individual leaves and side shoots. After determining the fresh weight, the plant samples were airdried for ^{14}C determination (<30°C) or stored at -20°C for extraction purposes. The soil samples were airdried (<30°C), weighed and homogenized for ^{14}C determination and desorption.

Detection of Radioactivity and Determination of Active Ingredient

^{14}C measurements were carried out for all samples by LSC (Packard, Tri-Carb^{R}, 460, 4530, 2500TR); solid samples were first combusted in the Packard 306 sample oxidizer. In order to determine the imidacloprid content in the plant samples, the plant material was extracted with methanol-water (80:20, v/v), the extract reduced to the aqueous fraction at 40 °C and this latter was then partitioned against ethyl acetate. Imidacloprid in the concentrated organic phase was analyzed by thin-layer radiochromatography (radio-tlc). The potential active ingredient availability in the dressing zone region close to the grain was determined by a two-stage desorption of 10 g soil with 0.01 M $CaCl_2$ solution in a ratio of 1:5. After concentrating the desorption solution, the imidacloprid fraction was detected by radio-tlc. Radio-tlc was carried out with a tlc-analyzer (Berthold, LB 2842) on silica gel plates ($60F_{254}$, Merck), developed in chloroform, acetone, glacial acetic acid and water (50:30:15:1, v/v/v/v). Imidacloprid was identified by comparing the R_f values with labelled (radio-tlc, autoradiography) and unlabelled (UV detection) reference substance.

Biotest with Aphids

Rhopalosiphum padi and *Macrosiphum avenae* were applied in cages to the youngest, completely developed leaf of each wheat plant, whether treated with imidacloprid or not, from the 4-leaf stage (14) once a week and the mortality rates assessed after 4 days.

RESULTS

Dressing Zone Formation

The distribution of the ^{14}C activity in the soil was characterized by an increasing ^{14}C translocation out of the dressing zone region close to the grain into the remaining dressing zone which was intensified by higher soil moisture contents (Table 1). With values of between 45 % (30 % WHC_{max}) and 29 % (40 % WHC_{max}), 195 days after sowing, a high proportion of the ^{14}C activity of the dressing zone remained in the region close to the grain, namely a maximum of 2.5 cm adjacent to and 2.5 cm below the caryopsis. Correspondingly, the concentration of active ingredient equivalents was *considerably higher* than in the remaining dressing zone, irrespective of the soil moisture content, with values of between 1.1 - 1.2 mg/kg soil (111 days after sowing) and 0.3 - 0.4 mg/kg soil (195 days after sowing). As a consequence of the increasing ^{14}C translocation the active ingredient equivalents increased in the remaining dressing zone to a maximum of 0.06 - 0.07 mg/kg soil (195 days after sowing).

Table 1. Radioactivity in the dressing zone close to the grain at various soil moisture contents. Radioactivity of the total dressing zone sample = 100 %.

Days after sowing	30 % WHC_{max}	40 % WHC_{max}	50 % WHC_{max}
111	94	96	72
153	79	58	62
195	45	29	34

Table 2. Desorbable imidacloprid in the dressing zone close to the grain at various soil moisture contents. Radioactivity in the dressing zone region close to the grain = 100 %.

Days after sowing	30 % WHC_{max}	40 % WHC_{max}	50 % WHC_{max}
111	62	61	58
153	46	41	41
195	27	23	22

The potential availability of imidacloprid in the soil, measured as the desorbable fraction of active ingredient in the ^{14}C activity of the dressing zone region close to the grain (Table 2), dropped to values of between 27 % (30 % WHC_{max}) and 22 % (50 % WHC_{max}) up to 195 days after sowing, due to a reduced desorbability of the ^{14}C activity in connection with declining fractions of active ingredient in the desorbed radioactivity.

Uptake, Distribution and Active Ingredient Concentrations in the Plant

Uptake and distribution of radioactivity at medium soil moisture

At 40 % WHC_{max}, the ^{14}C activity in the wheat shoot rose continuously up to 19 % of the radioactivity applied on average per caryopsis or 9.5 µg of active ingredient equivalents at full ripeness (91) (Fig. 1). However, the concentration of active ingredient equivalents in the shoot during tillering and shooting dropped from 14 mg/kg fresh weight (111 days after sowing) to 0.19 mg/kg fresh weight (195 days after sowing) due to an increasing difference between the incorporation of ^{14}C and growth of fresh weight (Fig. 1). Due to the loss of fresh weight during ripening, the concentration rose to 1.0 mg of active ingredient equivalents/kg fresh weight (260 days after sowing)(Fig. 1).

The major fraction of the ^{14}C activity taken up into the plants was always translocated into the shoot and distributed there in such a way that a gradient decreasing from the oldest to the youngest leaf arose for the concentration of active ingredient equivalents. The concentrations in the oldest, senescent leaves reached values of between 20 and 80 mg/kg fresh weight, whereas in the youngest, not completely formed leaves the concentrations were in part lower than 100 µg/kg fresh weight. The youngest leaves which were completely formed and physiologically active towards the end of shooting (45) represented an exception. In this case, the concentrations were on a uniform level between 0.12 and 0.18 mg of active ingredient equivalents/kg fresh weight. At the time of full ripeness (91), the concentrations of active ingredient equivalents in the straw and chaff (1.2 - 1.9 mg/kg dry mass) were much higher than in the grain (0.04 mg/kg dry mass). On macroautoradiographies of the wheat plants an inhomogeneous ^{14}C distribution within the individual leaves with high ^{14}C concentrations at the tips, edges and in the veins can be recognized, apart from the concentration gradient between various old leaves.

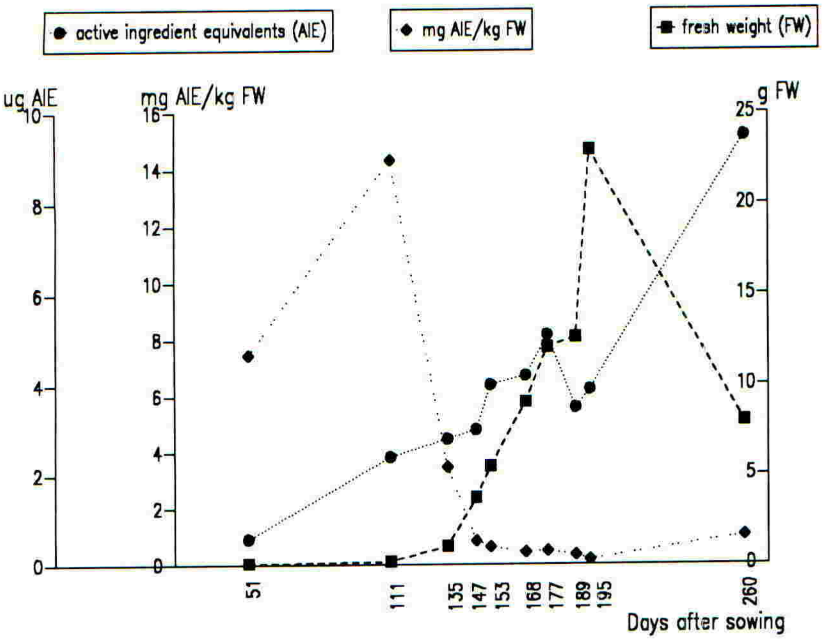


Fig. 1. Fresh weight (FW), active ingredient equivalents (AIE) and concentration of active ingredient equivalents in the wheat shoot at medium soil moisture content (40 % WHC_{max}).

Radioactivity uptake and concentrations of active ingredient at various soil moisture contents

Higher soil moisture contents caused an increased removal of ^{14}C from the caryopsis as well as reduced uptake in the plant (Table 3). Up to the end of shooting (45), between 22 % (30 % WHC_{max}) and 7.0 % (50 % WHC_{max}) of the ^{14}C activity applied per caryopsis was incorporated into the shoot. Since there was no influence of soil moisture content on the fresh weights of the wheat plants, the reduced ^{14}C uptakes at higher soil moisture contents also led to reduced concentrations of active ingredient equivalents in the shoot. As already described for 40 % WHC_{max}, the concentration during tillering and shooting dropped considerably at 30 and 50 % WHC_{max}.

The active ingredient concentrations in the wheat leaves declined considerably with increasing age of the leaves (Fig. 2) due to a decrease of the active ingredient fraction in the ^{14}C activity in the leaf. However, due to the pronounced ^{14}C concentration gradient decreasing from the oldest to the youngest leaf, the active ingredient concentrations in the older leaves were higher than those in the younger leaves at this date in spite of lower proportions of active ingredient. The influence of higher soil moisture content became apparent in the reduced concentrations of extractable imidacloprid (Fig. 2). This effect is largely based on the lower incorporation of ^{14}C into the shoot or the individual leaves, but also on reduced proportions of active ingredient in the ^{14}C activity in the leaf. 195 days after sowing, the imidacloprid concentration in the 8th leaf was between 12 µg/kg fresh mass (50 % WHC_{max}) and 81 µg/kg fresh mass (30 % WHC_{max}), whereas in the 10th leaf (flag leaf) the corresponding values amounted to 16 and 110 µg/kg fresh weight respectively.

Biotest

During the observation period (140 - 190 days after sowing), the insecticidal action against aphids proved to be independent of the soil moisture content, and 192 days after sowing amounted to at least 70 % against *M. avenae* and 98 % against *R. padi*, tested on the 8th - 9th leaf of the wheat.

Table 3. Radioactivity in the caryopsis, root and shoot at various soil moisture contents. Average radioactivity applied per caryopsis = 100 %.

Soil moisture content		Days after sowing		
		111	153	195
Caryopsis	30 % WHC_{max}	16	4.3	0.90
	40 % WHC_{max}	6.0	1.0	0.48
	50 % WHC_{max}	2.8	0.60	0.24
Root	30 % WHC_{max}	0.20	1.9	1.9
	40 % WHC_{max}	0.12	0.85	1.0
	50 % WHC_{max}	0.090	0.57	0.59
Shoot	30 % WHC_{max}	6.7	15	22
	40 % WHC_{max}	4.8	7.9	7.7
	50 % WHC_{max}	2.7	4.6	7.0

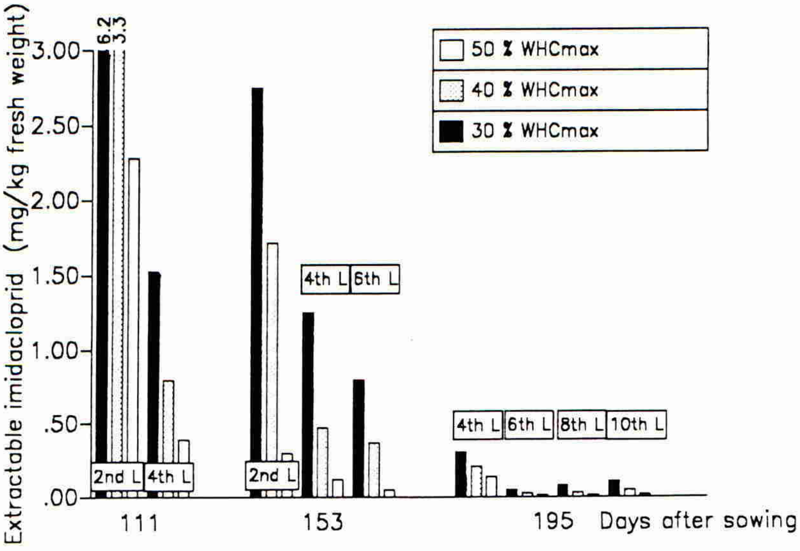


Fig. 2. Concentrations of extractable imidacloprid in the wheat leaves 111, 153 and 195 days after sowing.

DISCUSSION

A long-term protection against aphids for winter wheat by means of seed treatment requires both a sufficient subsequent supply of active ingredient from the soil or uptake into the plant as well as a distribution in the plant, by means of which effective concentrations of insecticide can be provided in all parts of the plant to be protected.

Although after seed treatment with [^{14}C]imidacloprid the uptake of ^{14}C-labelled compounds into the wheat plant continued even beyond the shooting stage (45) (Fig. 1) the subsequent delivery of ^{14}C or active ingredient took place with diminishing intensity. As a result, irrespective of the soil moisture level, a decrease in active ingredient equivalent or active ingredient concentrations in the plant resulted during tillering and shooting of the wheat. Higher contents of soil moisture reduced both the time and also the level of subsequent delivery into the plants (Table 3). These relations were also observed after the seed dressing of winter cereals with [^{14}C] triadimenol (Thielert, 1984; Schneider, 1988) and were based, amongst other aspects, on a declining potential plant availability of triadimenol in the soil, which occurred due to progressive dressing zone formation and the associated adsorption, as well as the increasing active ingredient degradation. With progressing plant development, a ^{14}C translocation also took place in the dressing zone of the winter wheat treated with [^{14}C]imidacloprid (Table 1) leading to a dilution of the ^{14}C or active ingredient equivalent concentrations in the soil. In studies on desorption behaviour Fritz (1992) determined that the non-desorbable fraction is increased by a factor of 1.5 to 2 after ageing of the imidacloprid. In fact, the desorbability of imidacloprid decreased considerably during the experiment (Table 2). The lower active ingredient availability at higher soil moisture levels can be attributed to the increased dressing zone formation corresponding to the relations described above and also to degradation of the active ingredient. The observation that in the experiment the fraction of fine roots in the dressing zone was increased with lower soil moisture levels indicated that the soil moisture does not merely influence the potential active ingredient availability but rather, via root growth, the subsequent delivery of active ingredient into the plant.

The distribution of ^{14}C activity in the wheat plant was typical of xylem-mobile substances which accumulate in the plant parts with the highest transpiration so that a redistribution of the active ingredient present in the plant in favour of the new growth probably did not take place. Furthermore, as the decreasing active ingredient concentrations with leaf age show (Fig. 2), imidacloprid is subjected to a rapid metabolism in the plant. These experimental results illustrate the necessity of a continuous subsequent delivery of active ingredient from the soil for sustained insecticidal action.

Measures for improving the utilization of active ingredient and extention of the residual action after the seed dressing of winter wheat with imidacloprid must be directed towards a higher potential active ingredient availability in the dressing zone as well as an intensified root penetration of the dressing zone. Under soil and climatic conditions which could lead one to expect a greater dressing zone propagation and active ingredient adsorption in the soil, it might be possible to improve the active ingredient uptake and subsequent delivery into the plant by a suitable formulation of imidacloprid for a slow release from the seed. Possibilities of promoting root development in the dressing zone region can be found in soil working measures, addition of nutrients or combination of pesticide active ingredient and root growth regulators.

REFERENCES

Fritz, R. (1992) Personal communication. Bayer AG, Crop Protection Development, Institute for Metabolism Research, D-51368 Leverkusen, .

Huth, W. (1990) Barley Yellow Dwarf - ein permanentes Problem für den Getreideanbau in der Bundesrepublik Deutschland? *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **42**, 33-39.

Schmeer, H.E.; Bluett, D.J.; Meredith, R.; Heatherington, P.J. (1990) Field evaluation of imidacloprid as an insecticidal seed treatment in sugar beet and cereals with particular reference to virus vector control. *BCPC, Pests and Deseases 1990, Proceedings* **1**, 29-36.

Schneider, M. (1988) Aufnahme von [^{14}C]Triadimenol über Korn und Wurzel nach Flüssigbeizung von Wintergerste: Einfluß von Bodenfeuchte und Saattermin auf Radioaktivitätsverteilung und Wirkstoffgehalt in Pflanze und Boden. Dissertation Universität Bonn. *Berichte der Kernforschungsanlage Jülich* Nr. **2253**.

Thielert, W. (1984) Aufnahme und Nachlieferung von [benzolring-U-^{14}C]Triadimenol über die Karyopse und aus den Beizhöfen nach Saatgutbehandlung von Wintergerste und Winterweizen mit einer Trockenbeizformulierung. Dissertation Universität Bonn. *Berichte der Kernforschungsanlage Jülich* Nr.**1958**.