Comm. Appl. Biol. Sci, Ghent University, 70/3, 2005 **91**

INDIVIDUAL AND COMBINED EFFECTS OF DOSAGES OF AZOXYSTROBIN AND EPOXICONAZOLE IN WHEAT

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ABSTRACT

The effects of single fungicide applications on *Mycosphaerella graminicola* (septoria leaf blotch) control and winter wheat yield were evaluated in field trials conducted in central Belgium between 2000 and 2004. Individual applications of 25, 50, 75 and 100% of the manufacturer's recommended dose rates of azoxystrobin and epoxiconazole, and all the combinations of these treatments, were made at GS 39 in 2001 to 2004 and at GS 59 in 2000. Disease assessments were made at growth stage 75, some 7–8 weeks after the last applications.

Between 2000 and 2003, no significant difference was observed for disease control between the products when applied alone. With regard to the dose responses, the differences between the recommended dose rates and the 50% reduced dosages were not important. In 2004, azoxystrobin was less effective than epoxiconazole. This was probably the result of strobilurin-resistant isolates of *M. graminicola* reaching an occurrence of 32% before fungicide application.

The combination of different dosages of azoxystrobin and epoxiconazole revealed that there was very little synergy between these products when applied in a single application. The combinations of these products were better than individual applications only when high dosages of both compounds were used.

INTRODUCTION

Strobilurin fungicides have been officially tested in cereal crops in Belgium since 1993 (Meeùs & Bodson, 1996) and have been available to Belgian farmers since 1996. Using a new mode of action, the inhibition of mitochondrial respiration by binding to the cytochrome bc1 enzyme complex (Gisi *et al.*, 2002), they were an important addition to the existing fungicide range which was based mainly on sterol biosynthesis inhibitors (SBI). The first years of use have shown that this chemical class of fungicides provides excellent disease control and high yield increases in winter wheat in Belgium (Meeùs & Bodson, 1996, 1997, 1998). The timing of applications of strobilurins and SBI fungicides during the winter wheat crop growth appears to work in the same way under field conditions (Bodson and Meeùs, 1997).

For cereal disease protection it has always been recommended that strobilurins should be used in combination with an SBI fungicide. These chemicals belong to different cross-resistance group and their association thus conforms to guidelines for limiting the development of fungicide resistance. It has also been shown in the laboratory that the strobilurins inhibit the germination and penetration growth of several plant pathogenic fungi, while

SBIs generally do not inhibit fungal growth until after initial infection (Godwin *et al.*, 1994; Gold & Leinhas, 1994). The association of curative and protective fungicide properties was thus viewed as a potential advantage.

In 2002 there was a sudden occurrence of QoI resistance in *M. graminicola* in Ireland and northern England (FRAC, 2002 and 2003). In Belgium the first QoI-resistant strains were also detected at the end of the cropping season 2002, but their occurrence remained lower than 2% (Amand *et al.*, 2003). The proportion of resistance strains increased in the following years, reaching 32% before fungicide applications in 2004 (Maraite *et al.*, unpublished data).

In this context, our study was conducted to examine the interaction between a strobilurin (azoxystrobin) and an SBI (epoxiconazole) fungicide under field conditions. The effectiveness of mixtures of these compounds was analysed on septoria leaf blotch (SLB), caused by *Mycosphaerella graminicola* (Fuckel) Schroeter in Cohn. (anamorph *Septoria tritici* Rob. Apud Desm.), because it is one of the main diseases in winter wheat in Belgium. The experiment was repeated over five successive growing seasons, from 2000 to 2004, in order to get varying natural disease pressure, but also to assess the practical effect of the multiplication of QoI-resistant strains.

MATERIALS AND METHODS

One field trial was conducted in central Belgium each year between the 1999/00 and 2003/04 growing seasons. The trials were established in fields where agricultural procedures were carried out according to standard practice in Belgian farming. In each trial, individual applications of 25, 50, 75 (not in 2002) and 100% of the manufacturer's recommended dose rates of Amistar (azoxystrobin 250 g/L, SC; Syngenta recommended dose: 1.0 L/ha) and Opus (epoxiconazole 125 g/L, SC; BASF recommended dose: 1.0 L/ha), and all the mixtures containing these different ratios of both compounds were made in a single-spray programme. The applications were carried out when the flag leaf ligule became just visible (GS 39) (Tottman, 1987) in 2001 to 2004 or when the ears had just emerged completely (GS 59) in 2000, using hand-held spray booms (Table 1).

Assessments of the development of *M. graminicola* were made between 10 and 16 July each year (Table 1). The tillers were selected at random from each plot and the percentage diseased area of each flag leaf (referred to as F1) and second leaf, just under F1 (F2) estimated. The rate of disease control by the fungicides was calculated in comparison with the disease level observed in the untreated control plots.

At harvest, the whole plot was harvested using a combine harvester for plot work. The grain moisture was determined by electrical conductance and capacitive reactance using a DICKEY-john tester and the yields were adjusted at 15% moisture content.

Table 1. Details of the experimental conditions for the winter wheat trials, 2000-2004

	2000	2001	2002	2003	2004
Location	Roux-Miroir	Hamme-Mille	Lonzée	Lonzée	Lonzée
Wheat varieties	Windsor	Ordéal	Claire	Claire	Napier
Sowing dates	27/10/1999	13/10/2000	16/10/2001	20/11/2002	22/10/2003
Sowing rates (kg/ha)	140	175	110	155	145
Plot sizes (m2)	54	19	16	16	16
Number of replicates	4	4	5	5	5
Fungicide applic. dates	3/06/2000	23/05/2001	23/05/2002	28/05/2003	27/05/2004
Spray volume (L)	400	300	200	200	200
Disease assessment dates	26/06/2000	27/06/2001	-	-	28/06/2004
	10/07/2000	11/07/2001	16/07/2002	09/07/2003	13/07/2004
Nber of tillers analysed/plot	25	15	10	10	10
Harvest dates	24/08/2000	14/08/2001	13/08/2002	1/08/2003	9/08/2004

The occurrence of QoI-resistant strains of *M. graminicola* was determined by spreading conidial suspensions of single cirrhi picked up from a pycnidium on an SLB lesion on potato dextrose agar (PDA) plates amended or not with 1μg/ml azoxystrobin and by further confirmation of the resistance level in microtitre plates on potato dextrose broth (PDB) amended with various concentrations of azoxystrobin, according to Amand *et al.* (2003). The analyses were made over the 2000-2004 period on the winter wheat experimental platform of Lonzée and the results were reported by Amand *et al.* (2003) for 2000 to 2002. From 2002 to 2004 the analysed samples were collected within 200 m of the trials involved in this study.

RESULTS

Septoria leaf blotch development

The five growing seasons between 1999/00 and 2003/04 were quite different for the development dynamics of *M. graminicola*. In mid-July, the levels of the disease on the two upper leaves from the untreated plots varied highly from year to year (Table 2). In 2000 the disease developed early and very severely. The application of fungicide at GS 59, on 3 June, was quite late and the disease was already in incubation on the upper leaves. In mid-July the second leaf (F2) was completely destroyed by the disease, even in the treated plots. In the four subsequent seasons the disease was less severe and developed later. The applications made at GS 39 were preventative on the two upper leaves. In 2004 the level of infection on F1 was too low to make an accurate evaluation of product effectiveness.
No other disease developed in the untreated plot of the trials, except for some brown rust (*Puccinia recondita*) in trials carried out in 2002 and 2003. At no time did this disease cover more than 5% of the leaf F1 area in the untreated plots.

able 2. Diseased area (%) of the flag leaf (F1) and the leaf just under F1 (F2) with isible infection of *M. graminicola* in plots untreated with fungicides.

	6 June		20-25 June		10-16 July	
years	F1	F2	F1	F2	F1	F2
2000	1	4	71	91	100	100
2001			0	4	20	45
2002			nd	nd	47	92
2003			nd	nd	16	70
2004			0	9	7	65

nd = no data

requency of QoI-resistant strains

'he first detection in Belgium of resistance to QoI fungicides in *M. graminiola* was reported from a monitoring of the winter wheat experimental platorm of Lonzée, about 200 m from the trials in this study (Amand *et al.*, :003). The resistant strains were all isolated from samples collected in plots reated with 1.0 L/ha of Amistar. In April 2003 and 2004, 1 and 32%, repectively, of the strains isolated from samples collected before fungicide pplications on this experimental platform were able to grow on media mended with 1 µg/ml of azoxystrobin (Table 3). In both cases the sampling or this QoI resistance monitoring was also made less than 200 m from the rials involved in this study.

'able 3. Proportion of strains of *M. graminicola* isolated from samples collected from :002 to 2004 on the experimental platform of Lonzée and able to grow on PDA or PDB mended with 1 µg/ml of azoxystrobin.

Season	Sample collected	Wheat variety of the sample	Number of strains tested	% of strains growing on 1 µg/ml of azoxystrobin
2002	July (Amistar)	Pulsar	382	1.3
2003	April (untreated)	Drifter	81	1.2
2004	April (untreated)	Drifter	91	31.8

)ose response of azoxystrobin and epoxiconazole

'he control of leaf blotch on F1 and F2 with the full rate of the two products 'aried from year to year, depending on disease pressure (Figure 1). In 2000 he products were applied curatively at GS 59 and the control of the disease n F1 remained lower than 40%. During the four other seasons the applicaions were at GS 39 and the control with a full dose of epoxiconazole (Opus) 'aried from 69 to 90% on F1 and from 55 to 90% on F2.

'rom 2000 to 2002 the control of septoria leaf blotch on F1 and F2 was quite imilar for the two compounds when at least 50% of the full rate was used. Vith a quarter dose the control was lower than 6% with both products on F1 n 2000, and the effectiveness of Amistar was consistently lower than for)pus in 2001 and 2002.

n 2003 no difference emerged between the products on F1. On F2 the different dosages of Amistar were, however, not as effective as Opus. This loss of ffectiveness of Amistar in comparison with Opus was confirmed in 2004 vhen the full rate of this product controlled only 31% of the disease on the second leaf, while the full rate of Opus still controlled 89%. Nevertheless, the dose response was unclear with Amistar.

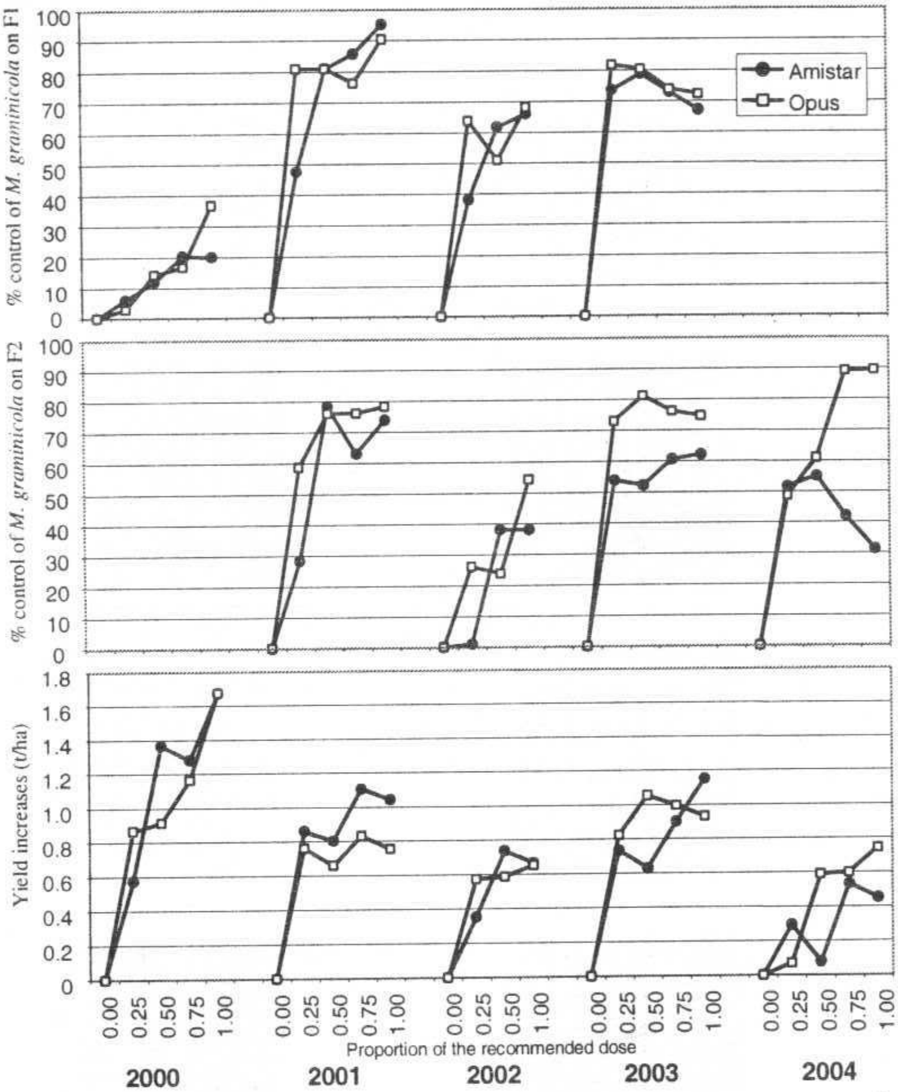

Figure 1. Effect of doses of Amistar (azoxystrobin 250 g/L, SC; recommended dose: 1.0 L/ha) or Opus (epoxiconazole 125 g/L, SC; recommended dose: 1.0 L/ha) applied once at GS 59 (2000) or at GS 39 (2001 to 2004) on the control of *M. graminicola* on the flag leaf (F1) and the second leaf (F2) and on yield.

From 2000 to 2003 the effects of the fungicide doses on yield were similar to the effectiveness on *M. graminicola*. In 2004, the difference in yield between the two products was, however, smaller than the difference in disease control.

From 2001 to 2003, it was found that a single application of 50% of the full recommended dose of both products was nearly as effective on SLB as the full dose. The differences in yield benefits were also very small, except for Amistar in 2003. For more curative applications under higher levels of infection in 2000, the effectiveness was more proportional to the dosages, up to the full dose.

Interactions between azoxystrobin and epoxiconazole

The interactions between azoxystrobin and epoxiconazole were analysed by applying mixtures containing different ratios of both compounds (Figure 2).

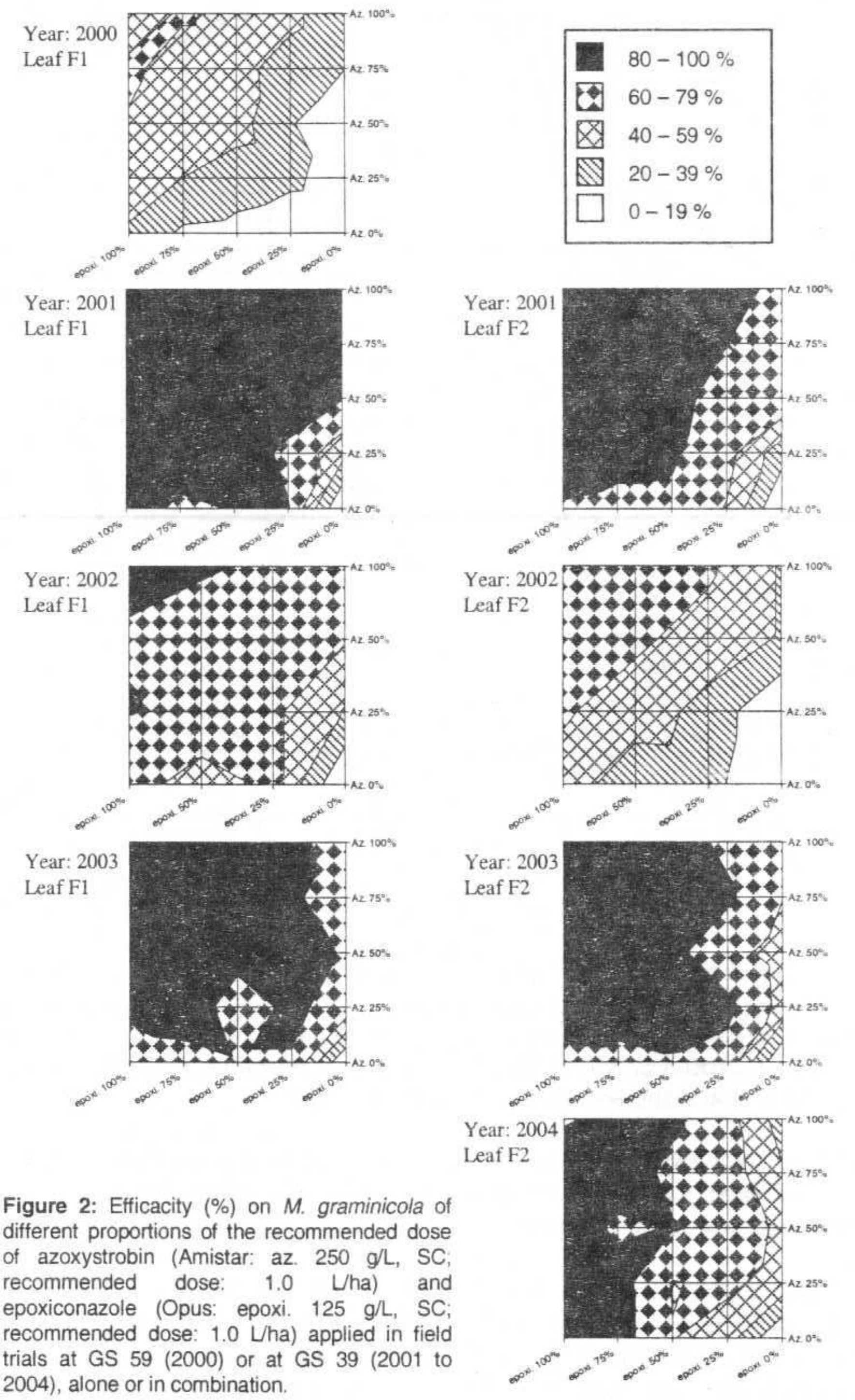

Figure 2: Efficacity (%) on *M. graminicola* of different proportions of the recommended dose of azoxystrobin (Amistar: az. 250 g/L, SC; recommended dose: 1.0 L/ha) and epoxiconazole (Opus: epoxi. 125 g/L, SC; recommended dose: 1.0 L/ha) applied in field trials at GS 59 (2000) or at GS 39 (2001 to 2004), alone or in combination.

From 2000 to 2002, the effectiveness observed on *M. graminicola* with these mixtures showed very little synergism between azoxystrobin and epoxiconazole. The interaction between these compounds was only additive. Such additive interaction was also observed for yield (Figure 3), mainly in 2000 and 2002, when the disease pressure was higher.
In 2003 the effectiveness of the two compounds on *M. graminicola* was less additive than during the three previous seasons. This was confirmed in 2004 when the effectiveness of the mixtures was almost proportional to the amount of epoxiconazole. However, no clear change in interaction between the two compounds was observed for yield during these two last seasons.

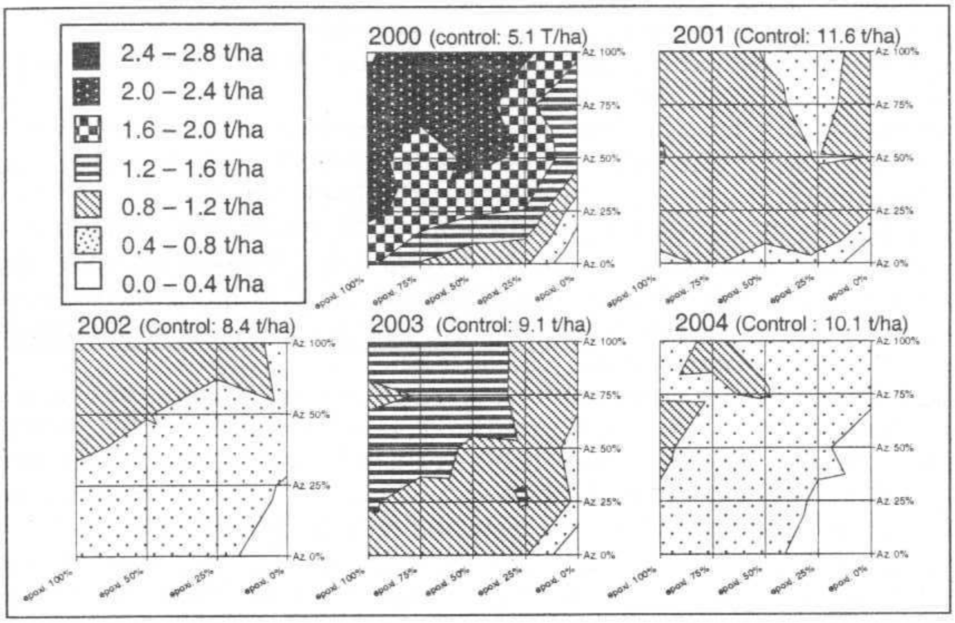

Figure 3. Yield increases following a single application in field trials at GS 59 (2000) or at GS 39 (2001 to 2004) of different proportions of the recommended dose of azoxystrobin (Amistar: az. 250 g/L, SC; recommended dose: 1.0 L/ha) and epoxiconazole (Opus: epoxi. 125 g/L, SC; recommended dose: 1.0 L/ha), alone or in combination.

DISCUSSION

All trials in this study included a comparison of fungicides and doses on cultivars susceptible to *M. graminicola*. However, the disease pressure varied greatly from season to season over the period 2000-2004 and the annual maximum yield benefit of the fungicide applications varied from 0.75 to 2.5 t/ha.
The dose-response curves of the single compounds showed that the control of *M. graminicola* and the resulting yield increases could be consistent with half of the manufacturer's recommended dose of azoxystrobin or epoxiconazole. This was consistently verified from 2001 to 2004 when the disease pressure was moderate and the products were applied at GS 39. Mercer and Ruddock (2005) recently reached the same conclusion in Ireland and they report similar findings from the 1990s in Germany and Denmark. Our trials revealed, however, that a quarter of dose can also achieve very good im-

provement, but less regularly from trial to trial. Moreover, with heavy disease pressure and when the fungicide application is made more curatively, as in our trial in 2000, the disease control and yield increased with the dose, up to the full dose.

From 2000 to 2002, before QoI resistance was detected in *M. graminicola* in Belgium, very few differences between Amistar and Opus were observed for the control of this disease and for yield. These results were not consistent with others showing that treatments with azoxystrobin gave a greater yield response than epoxiconazole (Bertelsen *et al.*, 2001). They were mainly unexpected in 2000, when the fungicides were applied more curatively, because the fungicidal effect of azoxystrobin is known to be stronger at an early stage of fungal development compared with epoxiconazole, which inhibited mycelial growth (Bertelsen *et al.*, 2001; Godwin *et al.*, 1994; Schöfl & Zinkernagel, 1997).

The results of our field trials with the mixtures containing all the combinations of 25, 50, 75 and 100% of the manufacturer's recommended dose of epoxiconazole and azoxystrobin consistently displayed additive effects. The synergetic activity between the two compounds under field conditions was very small. This is consistent with the interactions observed on spore germination and germ tube growth of pycnidiospores of *M. graminicola* between the azole fungicide cyproconazole and the strobilurin kresoxim-methyl (Stergiopoulos and De Waard, 2002).

In 2002 there was a sudden increase in resistance of *M. graminicola* to strobilurin fungicides in various European countries (FRAC, 2002, 2003). In Belgium, surveys for resistance in this pathogen have been made by Amand *et al.* (2003) since 2000, but the first strains able to grow on media amended with 1 µg/ml of azoxystrobin were detected only at the end of the 2002 cropping season. This confirms that the 5 years covered by this study include a real change in the sensitivity of this pathogen to strobilurins. In Lonzée, all the strobilurin applications made in 2003 and 2004 resulted in severe increases of the proportions of resistant strains (Maraite, unpublished data). In this context, our results over the last two seasons displayed a clear loss in the effectiveness of azoxystrobin on *M. graminicola* in comparison with epoxiconazole. In 2004, when 32% of the strains of *M. graminicola* isolated before fungicide applications were resistant to strobilurins, the effectiveness of azoxystrobin applied alone was still 31%, but the additive interactions with epoxiconazole appeared to be non-existent. It therefore appears unlikely that strobilurin fungicides will play a major role in *M. graminicola* control in the future. Nevertheless, the effects of the occurrence of QoI resistance on the disease control observed in our trials were not clearly translated into yield.

REFERENCES

AMAND O., CALAY F., COQUILLART L., LEGAT T., BODSON B., MOREAU J-M. & MARAITE H. (2003). First detection of resistance to QoI fungicides in *Mycosphaerella graminicola* on winter wheat in Belgium. Communications in Agricultural and Applied Biological Sciences, Ghent University, **68(4b)**:519-532.

BERTELSEN J.R., DE NEERGAARD E. & SMEDEGAARD-PETERSEN V. (2001). Fungicidal effects of azoxystrobin and epoxiconazole on phyllosphere fungi, senescence and yield of winter wheat. Plant Pathology, **50**:190-205.

BODSON B. & MEEÙS P. (1997). Intérêt et positionnement d'un traitement fongicide avant le stade épiaison sur froment d'hiver dans les conditions culturales de la Belgique. Med. Fac. Landbouww. Rijksuniv. Gent, **62/3b**:1009-1015.

FRAC (2002). QoI working group of FRAC. Minutes of a meeting on November 21st-November 22nd 2001. FRAC website: http://www.frac.info/qoi_wg.html.

FRAC (2003). QoI working group of FRAC. Minutes of a meeting on 13th-January 14th 2003. FRAC website: http://www.frac.info/qoi_wg.html.

GISI U., CHIN K.M., KNAPOVA G., KÜNG FÄRBER R., MOHR U., PARISI S., SIEROTZKI H. & STEINFELD U. (2000). Recent developments in elucidating modes of resistance to phenylamide, DMI and strobilurin fungicides. Crop Protection, **19**:863-872.

GODWIN J.R., YOUNG J.E. & HART C.A. (1994). ICIA 5504: Effects on development of cereal pathogens. *Proceedings of the Brighton Crop Protection Conference*. Farnham, UK: British Crop Protection Council, 259-264.

GOLD R.E. & LEINHOS G.M.E. (1994). Histological studies on the fungicidal activity of the strobilurin BAS 490 F. *Proceedings of the Brighton Crop Protection Conference*. Farnham, UK: British Crop Protection Council, 253-258.

MEEÙS P. & BODSON B. (1996). Résultats de trois années d'essais fongicides dans les céréales en Belgique avec les strobilurines. Med. Fac. Landbouww. Univ. Gent, **61/2a**:395-404.

MEEÙS P. & BODSON B. (1997). Protection fongicide du froment d'hiver en Belgique: Incidence des strobilurines en fonction du stade d'application. Med. Fac. Landbouww. Univ. Gent, **62/3b**:1117-1127.

MEEÙS P. & BODSON B. (1998). Optimising strobilurine fungicide uses in a reasoned protection of winter wheat. Med. Fac. Landbouww. Univ. Gent, **63/3b**:1023-1027.

MERCER P.C. & RUDDOCK A. (2005). Disease management of winter wheat with reduced doses of fungicides in Northern Ireland. Crop Protection, **24**:221-228.

STERGIOPOULOS I. & DE WAARD M.A. (2002). Activity of azole fungicides and ABC transporter modulators on *Mycosphaerella graminicola*. J. Phytopathology **150**:313-320.

SCHÖFL U.A. & ZINKERNAGEL V. (1997). A test method based on microscopic assessments to determine curative and protectant fungicide properties against *Septoria tritici*. Plant Pathology, **46**:545-556.

TOTTMAN D.R. (1987). The decimal code for the growth stages of cereals with illustrations. Annals of Applied Biology **110**:441-454.