Data from monitoring for Fungicide Resistance in cereals 2012

Septoria tritici isolates sensitivity to triazoles in Denmark, Sweden, Latvia and Lithuania

Data from NORBARAG Trial

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Sensitivity in the Septoria population to triazoles in Sweden and Denmark 2012

Summary

Monitoring work

Monitoring for triazole resistance in the *Septoria tritici* population was carried out by Aarhus University, Department of Agroecology, Flakkebjerg. The project was carried out in collaboration with the Knowledge Centre for Agriculture, Denmark and the Swedish Board of Agriculture, Sweden, who together provided the samples with *Septoria tritici* from trials or farmers' fields. In total, samples from 18 Danish localities and 35 Swedish localities were investigated. Five samples from Latvia and Lithuania were also included in the work provide by Ilze Priecule (Latvia) and Roma Semaskiéné (Lithuania). The test was carried out as a bioassay, using isolates from leaf samples and cultured before screening on different concentrations of fungicides. A further sensitivity testing was also carried out for difenoconazole, boscalid and folpet on a randomly chosen number of isolates from Sweden and Denmark. The report also provides data from field performances using triazoles for control of septoria leaf blotch.

The report also includes a summary of data from 5 field trials carried out in winter wheat testing different fungicides and their impact on control of Septoria as well as their impact on selection of different R types of Septoria.

Denmark

In Denmark epoxiconazole is most commonly used for control of *Septoria tritici* blotch, either alone or in combinations with other triazoles or boscalid. From 2012 40 isolates from 18 localities were tested in bioassays.

In comparison with previous years there has not been any decrease in sensitivity to epoxiconazole; if anything sensitivity has increased. The level of insensitivity to prothioconazole is still high and the whole population has shifted and become less sensitive. The average EC_{50} values in 2012 in Denmark were 0.3 ppm and 10.9 ppm for epoxiconazole and prothioconazole, respectively. Previously, EC_{50} for epoxiconazole was as high as 1.4 ppm in 2010 and as such the value has decreased. This indicates that although shifting has taken place, the sensitivity seems to have stabilised. Prothioconazole was included in the Danish testing in 2009 for the first time when the EC_{50} value was 0.3 ppm. Since then,the EC_{50} value has increased to around 10 ppm, and today almost none of the very sensitive isolates are left. Generally the field performances from both epoxiconazole and prothioconazole were satisfactory in the 2012 season. As seen in previous years a slightly better field performance from epoxiconazole was also seen in 2012 compared with prothioconazole.

Sweden

In Sweden prothioconazole is the main triazole used for control of *Septoria tritici* as epoxiconazole is not authorised. 211 isolates from 35 localities were tested in 2012. In comparison with previous years the changes in sensitivity to prothioconazole have been noticeable. The average EC_{50} values in 2012 in Sweden were 0.36 ppm and 13,3 ppm for epoxiconazole and prothioconazole respectively. EC_{50} for prothioconazole has increased from 1.5 ppm in 2008 to 13,3 ppm in 2012. This indicates that a shifting has taken place. The sensitivity to prothioconazole initially changed to a bimodally shaped curve, but today it is again a monomodal population. Today 80% of the localities had average EC_{50} values higher than 10 ppm for prothiconazole. These localities were evenly distributed across the country. The resistance factor for prothioconazole was high and varied from 23 to 157. The EC_{50} value for epoxiconazole was 0,36 ppm for 2012, which was slightly lower compared with values from previous years. The resistance factors were approximately 18, which was clearly lower than the resistance factors for prothioconazole. As in Denmark, epoxiconazole sensitivity in 2012 had increased compared to the previous years, indicating a stabilisation of the population. The values indicate no clear signs of cross resistance between the two triazoles. The field performances in trials carried out in Sweden in 2012 show slightly lower levels of control compared with the previous year. The number of trials is, however, still too limited to conclude whether or not the field performances have been influences.

Lithuania and Latvia

Few isolates (24) originating from 2012 were tested from these two countries. In both Latvia and Lithuania the average sensitivity was around 0.13 ppm for epoxiconazole and 7.3 ppm for prothioconazole. The data show a similar trend as seen for Denmark and Sweden where the sensitivity to epoxiconazole has increased. The sensitivity to prothioconazole was in line with or increased compared with 2011. There is a long tradition for using triazole products in the Baltic countries. Field performances in the Baltic countries from triazoles have generally been satisfactory.

Difenoconazole, boscalid and folpet

In total 73 isolates from Sweden and Denmark were tested for sensitivity to difenoconazole, boscalid and folpet. EC_{50} values for difenoconazole was very low and resistance factors around 15 in line with the level for epoxiconazole. Resistance factors for boscalid was around 1 indicating no shifting has taken place. Resistance factors for folpet was strangely very variable for isolates and several isolates gave relatively high EC50 values despite the fact that the product is multisite inhibitor and has not been authorised yet. Following this rather unexpected finding for folpet some isolates were shared with Rothamsted research station (UK). They found the isolates to be much more sensitive and less variable in their test compared with the Danish test. Investigations are ongoing in order to check what could have gone wrong in the Danish testing of folpet.

Characterisation of the Septoria populations (by Bayer, Syngenta and BASF)

In a mutual Septoria trial carried out by NORBARAG 6 different control strategies were tested in 5 trials (2 DK, 2 SE and 1 LI). Efficacy and yield were assessed and leaves were sampled to test if different strategies will select for different CYP51 mutations. The leaves analysed by Bayer, BASF and Syngenta showed a major dominance of I381V, low to moderate amounts of G379, A136 and C136 and only minor amounts of A134 and T524. Most treatments did not clearly select for any pattern, but Sportak seems in 1-2 trials to select for A136. In one trial Opus and Proline also selected quite strongly for A134 and V136.

The level of Septoria in the trials were low to moderate. The field control from the different strategies was generally good with a slight advantages to Aviator. In the Danish trials Proline was inferior to other treatments, but this was not similarly seen at the localities in Sweden and Lithuania.

Method

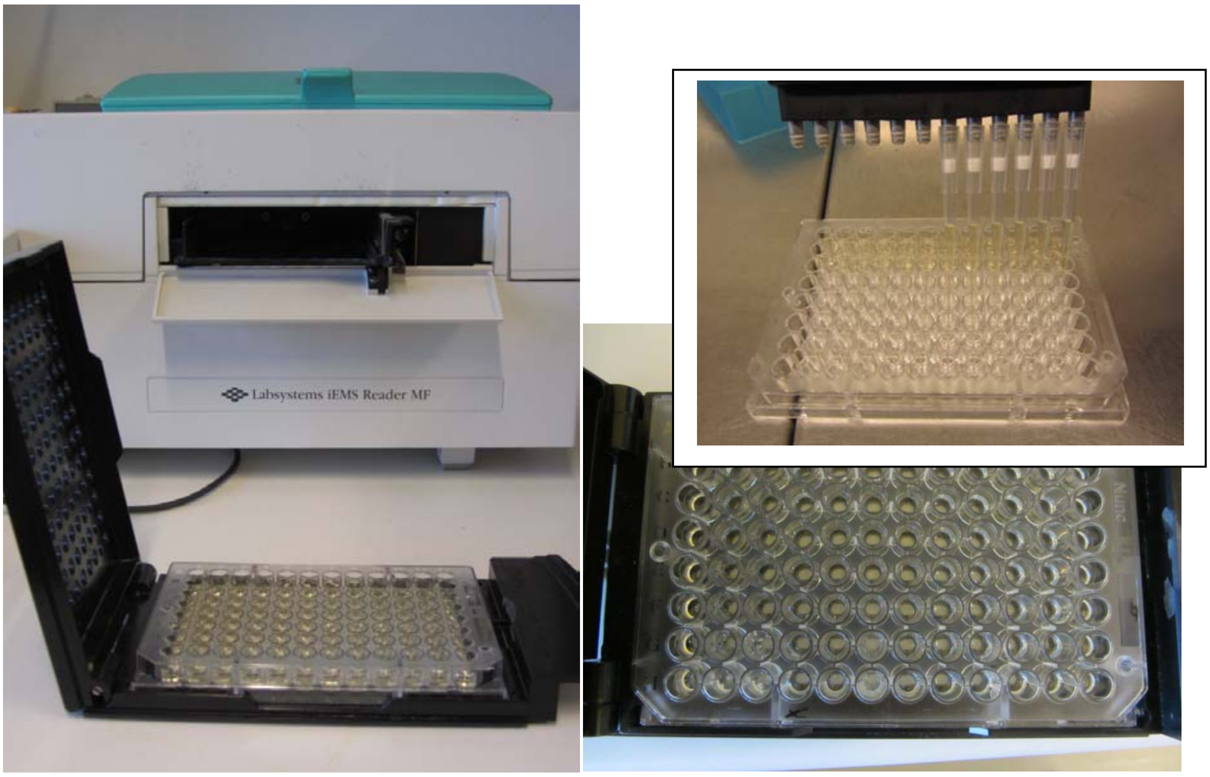
Septoria tritici

Around gs 71-75 leaf samples with 20-30 leaves were picked and sent to Flakkebjerg. On arrival the samples were dried. The samples were rehydrated and placed in boxes with wet filter paper. After 24 hours, the leaves were checked for spores. If no spores were observed, the leaves were incubated for a further 24h. The spores were transferred to PDA plates with 0.01% streptomycin and 0.01% rifampicine for propagation.

***In vitro* test**

Sensitivity towards epoxiconazole and prothioconazole was estimated on the basis of pure cultured isolates aiming at 10 isolates per locality. A spore suspension containing 2.5 x 10^4 spores/ml of each fungal isolate was produced from 5-day-old potato dextrose agar-grown vegetative *Septoria tritici* spores and transferred to a microtitre plate with 7 different concentrations of epoxiconazole (10, 3.333, 1.111, 0.37, 0.123, 0.041, 0.013 and 0) or prothioconazole a.i. (30, 10, 3.333, 1.111, 0.37, 0.123, 0.041 and 0) mixed with potato dextrose broth. Two replicates of each isolate were tested. The reference isolates S27 (WT) and RL2 (D. M. Hollomon, Long Ashton Research Station, UK), and IPO 323 were also included on one plate per run in order to ensure correct conditions. The microtitre plates were incubated at 22°C in the dark for 6 days, after which per cent growth was assessed relative to control by first measuring absorbance on Labsystems IEMS ELISA-reader and then converting into per cent inhibition. EC_{50} values were estimated by means of Graphpad prism software. Further retesting is needed if data should lead to a more exact value.

In the last testing for sensitivity to other fungicides 7 different concentrations of difenoconazole and boscalid were used (10, 3.333, 1.111, 0.37, 0.123, 0.041, 0.013 and 0) and for folpet (30, 10, 3.333, 1.111, 0.37, 0.123, 0.041 and 0) were used mixed with potato dextrose broth.



Microtitre plate with fungicide gradient and spore growth.

Results from Denmark

Sensitivity to epoxiconazole

The disease level of Septoria leaf blotch in 2012 was moderate to severe depending on the area investigated. In Jutland attacks were generally more pronounced than in Sealand. In 2012, samples were collected from 18 different localities but in total only 40 isolates were tested. Isolation of spores were difficult due to rain during the time of sampling, so from most localities only few isolates were tested.

The average EC_{50} value for epoxiconazole was 0.3 ppm, which was pronouncedly lower than in 2011. They sensitivity varied from 0.02 to 1.2 ppm. The reference isolate IPO 323 has an EC_{50} value of 0.02 ppm providing a variation in resistance factors between 1 and 19 for the tested localities. Compared to both 2010 and 2011 the data from 2012 showed a drop in EC_{50} values. The variation across sites was relatively small (Table 1).

Figure 1 shows the frequency of isolates belonging to the different sensitivity groups. Compared with previous years there seems to have been a stabilisation in sensitivity.

Table 1: Summarizing results from Danish *Septoria tritici* monitoring in 2012. EC_{50} for epoxiconazole and prothioconazole for Danish localities.

		Isolates	Epoxiconazole		Prothioconazole	
	Locality	Number	EC_{50}	R factor	EC_{50}	R factor
Gefion	1	1	0.02	1	0.03	1
Gefion	2	2	0.28	14	1.6	11
Kolding	3	2	0.25	13	0.31	2
Fyn	4	7	0.42	21	7.11	47
Åkirkeby	5	6	0.30	15	8.6	57
Årslev	6	1	0.20	10	20	133
Snogbæk	7	3	0.23	12	8.1	54
Trige	11	1	0.20	10	20	133
Landbo Limfjorden	12	1	0.20	10	20	133
Djursland Landboforening	14	1	0.35	18	20	133
Ytteborg	16	3	0.38	19	20	133
Ytteborg	17	2	0.36	18	10.6	71
LRØ	18	1	0.25	13	20	133
Jysk landbrugsrådgivning	20	4	0.26	13	12.8	85
Høve	23	2	0.35	18	20	133
Landbo Nord	24	1	0.20	10	20	133
Landbo Thy	25	1	0.37	19	10	67
Dalmose	27	1	0.20	10	12	80
		40	0.30	13	10.9	86

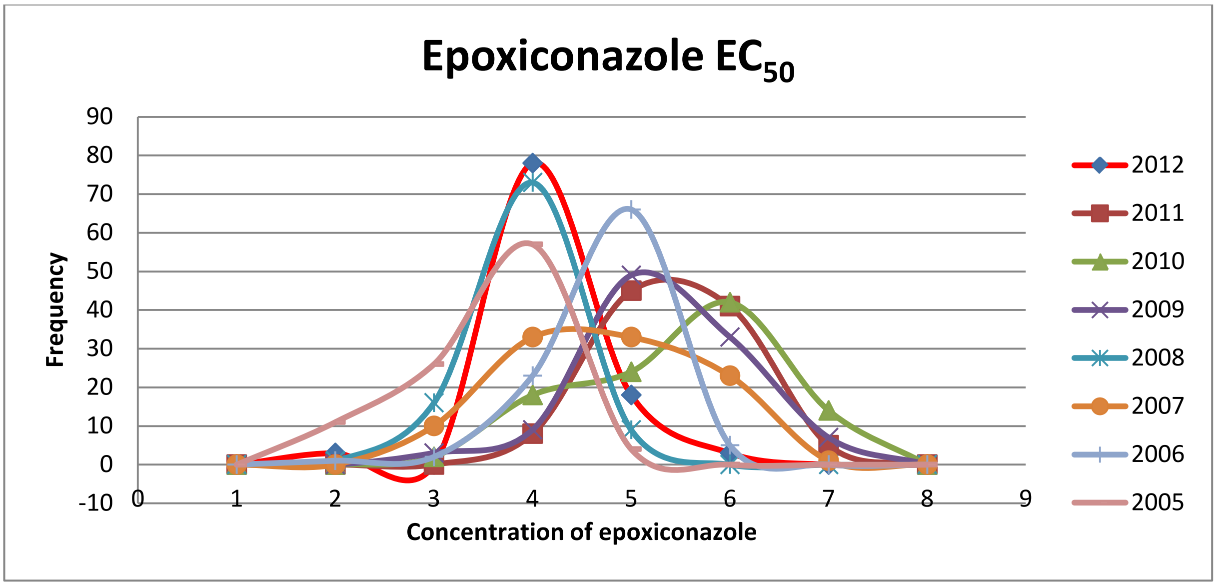
Sensitivity to prothioconazole

Based on 40 isolates from 2012 the average EC_{50} value to prothioconazole was 10.9 ppm (Table 1), Isolates varied in sensitivity between 0.03 and 20 ppm. With a few exceptions similar sensitivity was seen at most localities (Table 1). When the reference isolate IPO323 was used with a EC_{50} value of 0.15, the resistant factor varied from 1 to 133. Screening for sensitivity to prothioconazole in Denmark has taken place for 4 seasons 2009-2012. Sensitivity towards prothioconazole has declined noticeably compared to 2009 and 2010. Bimodal curves have changed significantly to the right indicating major shifting in the population (Figure 1). Average data from all years of monitoring are shown in Table 2.

Per cent control of *Septoria tritici* under field conditions was tested in several trials in 2012. Data in Figure 2 show the average control of *Septoria tritici* on flag leaves at gs 75-77 in trials originating from 1992 to 2012 with fungicides applied at gs 32-33 & 51-55. The field performance has not been clearly influenced by shifting taking place over the years. Comparison of efficacy levels from epoxiconazole and prothioconazole over the years has similarly been stable although prothioconazole in all years has provided approximately 10% less control compared with epoxiconazole.

Table 2: List of Danish EC_{50} values for epoxiconazole and prothioconazole assessed on *septoria tritici* in Denmark. No of isolates are given in brackets.

Year	EC_{50} epoxiconazole	R factor	EC_{50} prothioconazole	R factor
2005	0.12 (47)	2	-	
2006	0.57 (180)	10	-	
2007	0.77 (140)	13	-	
2008	0.17 (88)	3	-	
2009	0.7 (96)	12	0.7	7
2010	1.4 (54)	23	4.4	29
2011	1.33 (85)	22	11.2	74
2012	0.30 (40)	15	10.9	72
Wild type IPO323	0.02		0.15	



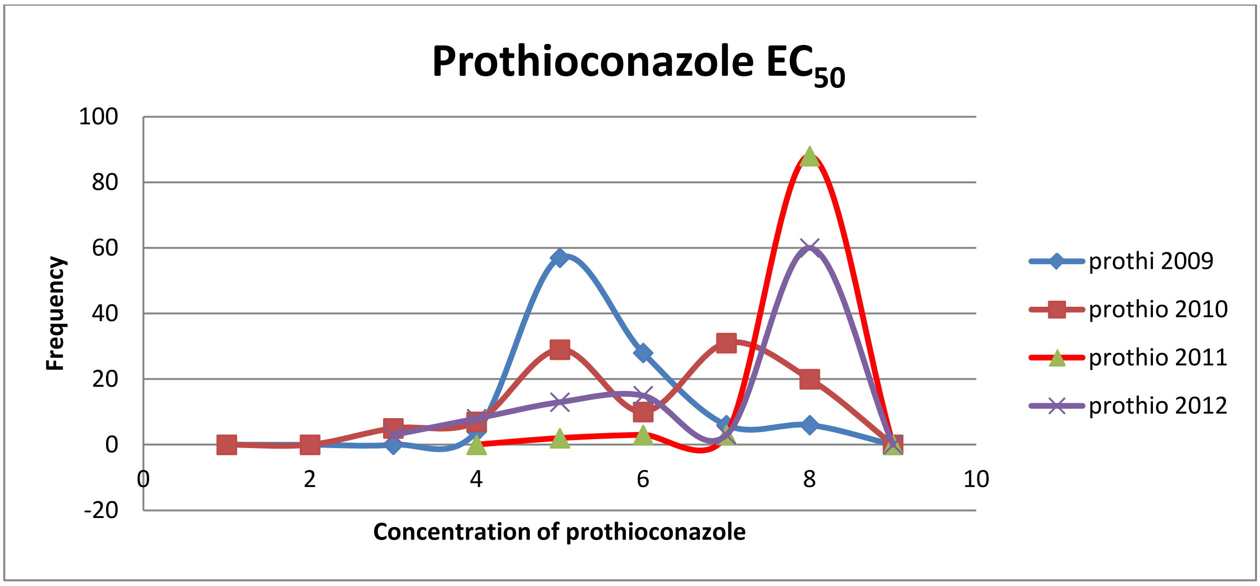
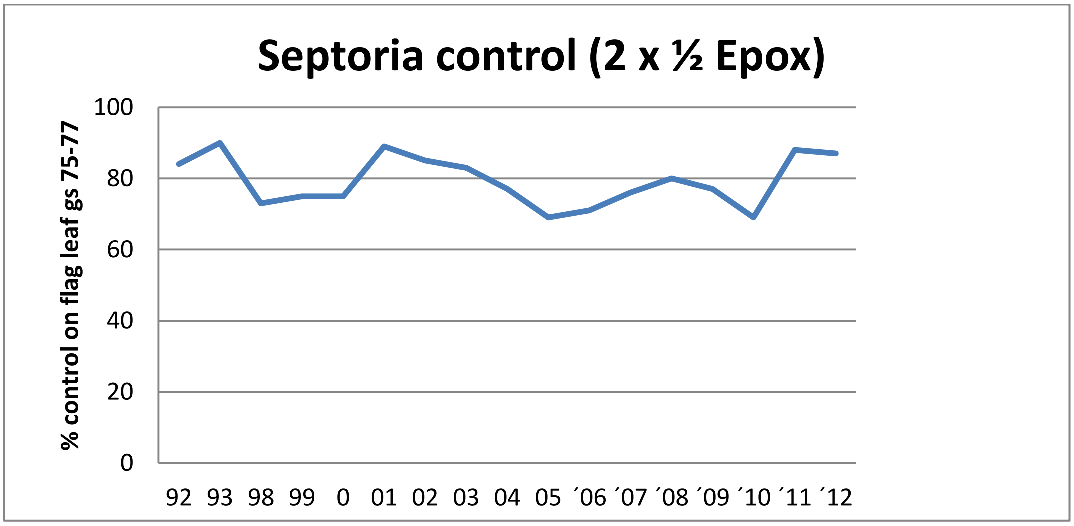
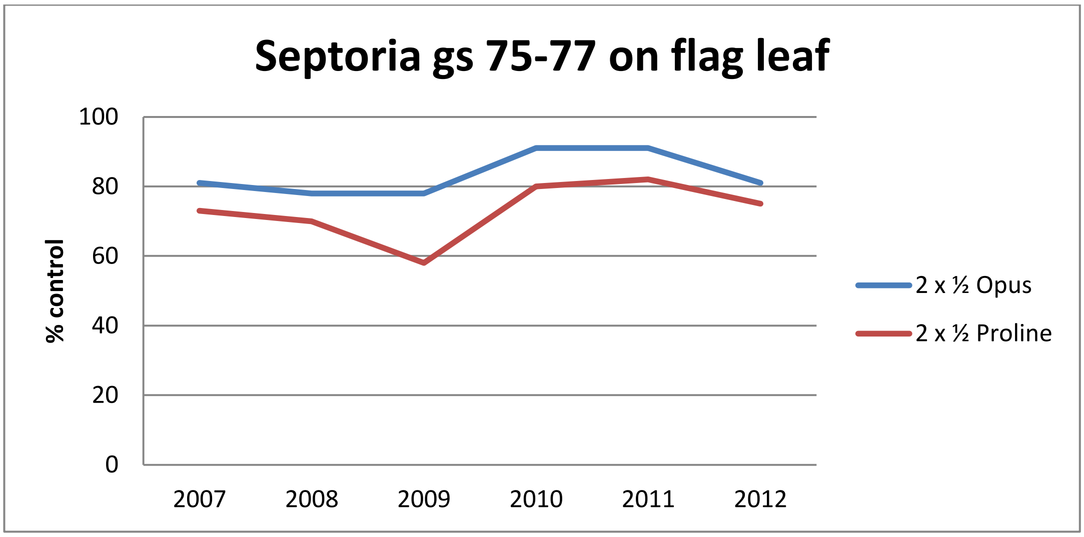


Figure 1: Distribution of sensitivity to epoxiconazole and prothioconazole - analysing Danish isolates from 8 years using epoxiconazole and 4 years with prothioconazole.

epoxi	<0.01	0.01-0.033	0.033-0.1	0.1-0.33	0.33-1,0	1.0-3.33	3.33-10	>10
prothio	0,01-0.03	0.03-0.1	0.1-0.33	0.3-1.0	1.0-3.3	3.3-10	10-30	>30
Classes X-aks	1	2	3	4	5	6	7	8



		% attack on flag leaf in untreated at gs 75-55	
Year	No. of trials	Untreated	% control
2006	16	65.9	71
2007	6	95	76
2008	3	11.4	80
2009	4	37.2	77
2010	7	61.7	69
2011	11	45.5	88
2012	8	16.7	87



	No. of trials	2 x ½ Opus	2 x ½ Proline
2007	4	81	73
2008	1	78	70
2009	4	78	58
2010	3	91	80
2011	6	91	82
2012	6	81	75

Figure 2: Per cent control of *Septoria tritici* under field conditions 1992-2012. Average of trials with 2 fungicides applied at gs. 31-32 & 51-55.

Results from monitoring in Sweden

Sensitivity to prothioconazole

The disease level of *Septoria tritici* blotch in 2012 varied across the country. In 2012 samples were collected from 35 different localities and in total 211 isolates were tested for sensitivity to prothioconazole and epoxiconazole (Table 3).

The average EC_{50} value to prothiconazole was 13,3 ppm. They varied from 0.01 to approx. 20 ppm. The reference isolate had sensitivities of 0.15 (IPO323), giving resistance factors varying from 23 to 167, indicating that over the years a significant change in sensitivity has taken place. Isolates with high levels of EC_{50} values could be found in all regions of Sweden (Figure 5). 80% of all localities had an average EC_{50} value higher than 10 ppm prothioconazole.

Sensitivity to epoxiconazole

In 2012 samples were collected from 35 different localities, and in total 211 isolates were similarly tested for sensitivity to epoxiconazole.

The average EC_{50} value to epoxiconazole was 0.36 ppm. The EC_{50} values varied for the localities between 0.2 and 1.7 ppm. The reference isolates IPO 323 had a sensitivity of approximately 0.02 ppm leaving the resistance factor to vary between 2 and 25 for the different localitites, indicating that over the years a minor change in sensitivity has taken place since the start of the triazole-era. In 2012 only 3% of the isolates had an EC_{50} value higher than 1 ppm. In 2011 this figure was similarly 29% of the isolates.

Figure 3 shows the distribution of isolates belonging to the different sensitivity groups. For prothioconazole the curve has been pushed to the right compared to 2010 and 2011, when a major shifting took place. The most sensitive isolates are almost now completely lost.

For epoxiconazole there seems to have been a minor decrease in sensitivity compared with earlier seasons. The resistance factor is lower for epoxiconazole compared with prothioconazole. Even though epoxiconazole is not authorised and used in Sweden, data indicate that selection goes on for this product also when prothioconazole is used.

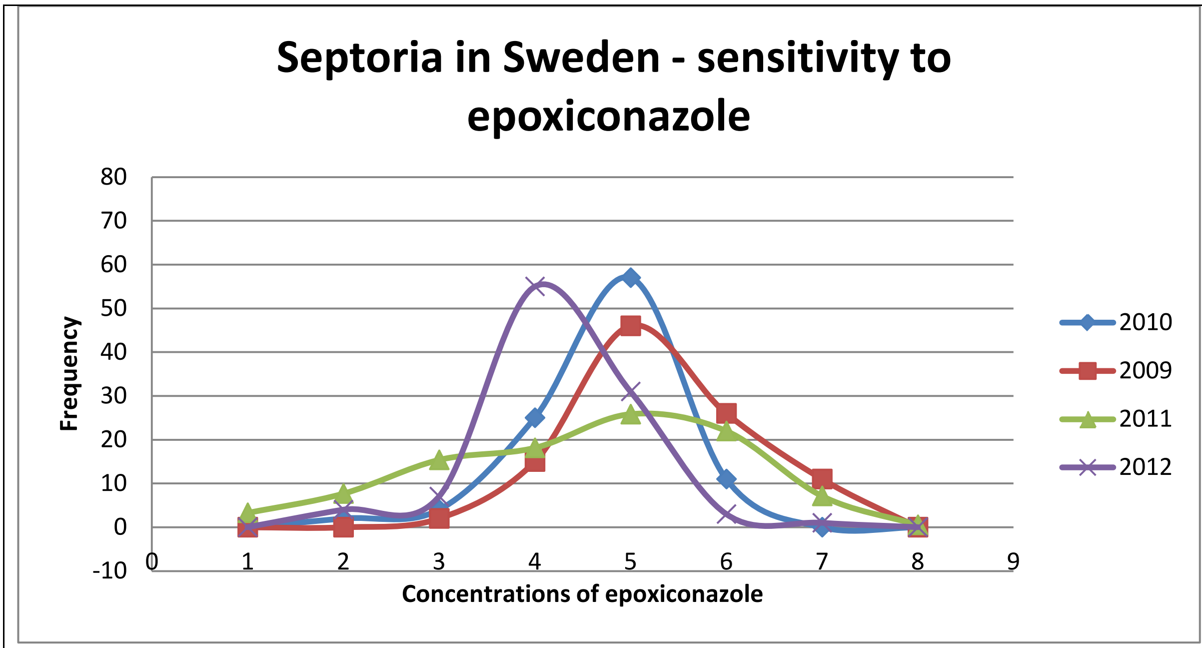
Average data from all years of monitoring in Sweden is shown in table 4.

Table 4: List of Swedish EC_{50} values for epoxiconazole and prothioconazole assessed on *septoria tritici* in Sweden. No of isolates are given in brackets.

Year	EC_{50} epoxiconazole	R factor	EC_{50} prothioconazole	R factor
2008	-	-	1,5 (55)	11
2009	-	-	3,6 (101)	24
2010	0,63	13	6,6 (131)	44
2011	1,0	16	7,8 (166)	52
2012	0,36	18	13,3 (211)	89
Wild type IPO323	0.02		0.15	

Table 3: Summarizing results from Sweden from 2012. EC_{50} for epoxiconazole and prothioconazole for Swedish localities in 2012.

			Prothioconazole			Epoxiconazole		
Sweden		No. of		IPO323	S27		IPO323	S27
	Locality	isolates	Ave. EC_{50}	R factor		Ave. EC_{50}	R factor	
2	Skarpenberga/Søderkøbin	6	13.5	113	68	0.195	10	10
3	Ystad/Norrkøping	5	18	150	90	0.294	15	15
4	Åbylund/Ledberg	3	16.7	139	84	0.2	10	10
5	Helleberga/Klockrike	3	13.3	111	67	1.6	80	80
6	Bobergs/Fornåsa	5	8.5	71	43	0.2	10	10
7	Forsa/mantorp	8	2.7	23	14	0.14	7	7
8	Forsby	3	6.2	52	31	0.03	2	2
9	Lidkøping	6	10.3	86	52	0.2	10	10
10	Lidkøping	6	3.2	27	16	0.25	13	13
11	Glættestorp	7	15.3	128	77	0.3	15	15
13	Bjertop	4	12.1	101	61	0.35	18	18
14	Sunnersberg	5	18	150	90	0.31	16	16
15	Lundsbrunn	6	20.8	173	104	0.43	22	22
16	Olofstorp	6	14.2	118	71	0.23	12	12
17	Grimskullen	6	14.2	118	71	0.3	15	15
18	Skofteby	9	12.7	106	64	0.3	15	15
19	Stockagården	5	9.4	78	47	0.198	10	10
		93	12,3	103	62	0,33	16	16
20	Eke	6	15.9	133	80	0.26	13	13
21	Hassleholm	5	19.4	162	97	0.46	23	23
22	Vara	3	15.3	128	77	0.40	20	20
23	Brunnby, Västerås	5	13.9	116	70	0.21	11	11
24	Tibble Västerås	4	9.5	79	48	0.17	9	9
25	Giresta Øresundsbro	7	10.7	89	54	0.41	21	21
26	Mycklinge Västerås	9	11	92	55	0.17	8	8
27	Angeby Uppsala	9	12.7	106	64	0.32	16	16
	Ave. mid Sweden	48	12,7	106	63,5	0.29	15	15
1	Kalmar	13	9.5	79	48	0.25	13	13
al1	Smedstorp	2	20	167	100	0.27	14	14
al2	Ønnestad1	10	18.2	152	91	0.3	17	17
al3	Ønnestad2	8	10.4	87	52	0.2	12	12
al4	Borreby	6	18.3	153	92	0.3	17	17
al5	Nyvång	4	12.5	104	63	0.5	26	26
al6	Nyboholm	5	17.0	142	85	0.5	25	25
al7	Lille Bøslid	9	9.8	81	49	0.2	12	12
al8	Trelleborg	4	16.3	135	81	1.7	83	83
al9	Skivarp Ystad	9	11.1	93	56	0.4	20	20
	Ave. south Sweden	70	14,3	119	72	0.47	24	24
	Ave. Sweden	211	13,3	111	67	0.36	18	18



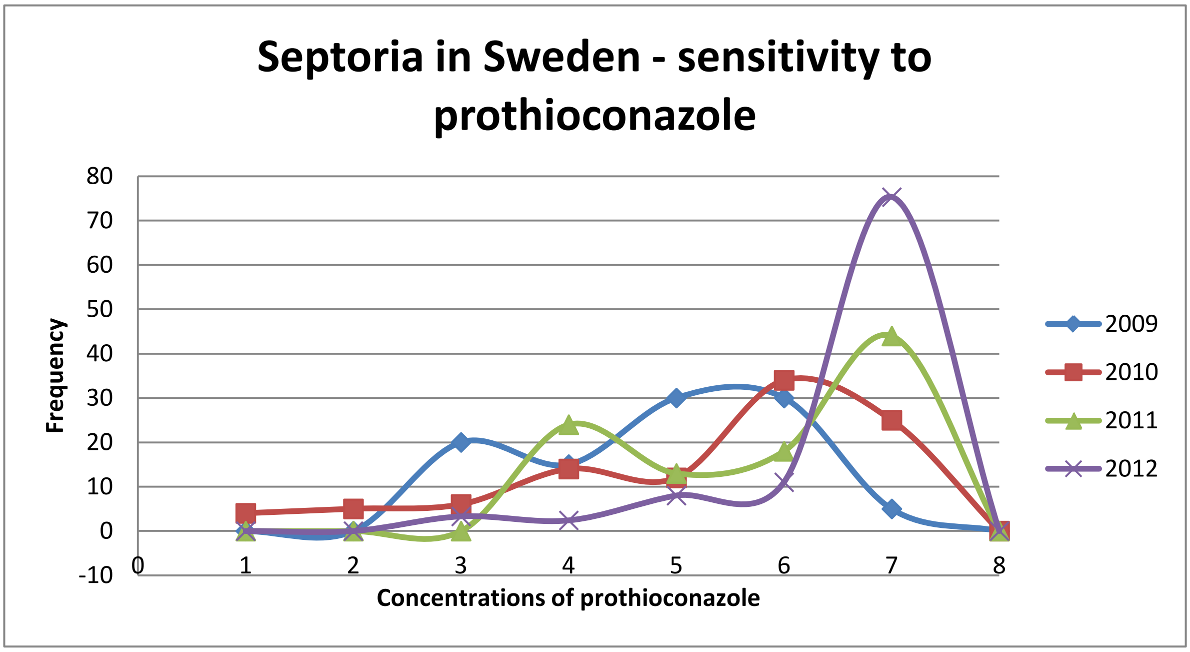
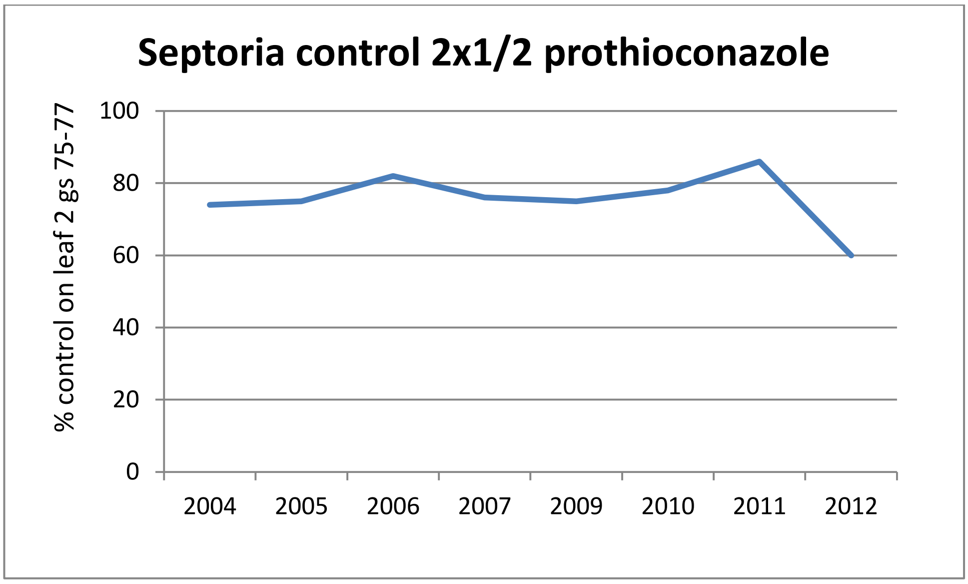


Figure 3: Distribution of septoria's sensitivity to prothioconazole and epoxiconazole analysing Swedish isolates from different years.

epoxi	<0.01	0.01-0.033	0.033-0.1	0.1-0.33	0.33-1,0	1,0-3,33	3,33-10	>10
prothio	0,01-0,03	0,03-0,1	0,1-0,33	0,3-1,0	1,0-3,3	3,3-10	10--30	>30
classes	1	2	3	4	5	6	7	8

Percent control of *Septoria tritici* under field conditions was tested in several trials in 2012. Data in Figure 4 show the average control of *Septoria tritici* on leaf two at gs 75-77 in trials originating from 2004 to 2012 with fungicides applied at gs 37-39 & 55-59. The field performance has not been clearly influenced by shifting taking place over the years and generally the field performances from prothioconazole was satisfactory in the 2012 season. Some trials carried out in 2012 show slightly lower levels of control compared with the previous year, but the number of trials is, however, still too limited to conclude whether or not the field performances have been influences.



		% attack on leaf 2 in untreated at gs 75-77	
Year	No. of trials	Untreated	% control
2004	4	21	75
2005	7	13	75
2006	4	17	82
2007	12	51	76
2009	6	36	75
2010	7	59	78
2011	8	46	86
2012	5	16	60

Figure 4: Per cent control of S*eptoria tritici* under field conditions 1992-2012. Average of trials with 2 fungicides applied at gs. 31-32 & 51-55.

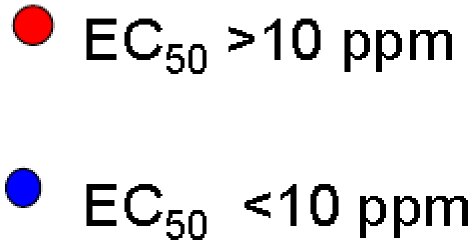
	2010 26 localities 
	2011 26 localities
	2012 35 localities

Figure 5: Map of Sweden showing the distribution of sites with prothioconazole EC_{50} values higher and lower than 10 ppm.

Results from Lithuania and Latvia

The attack of *Septoria tritici* blotch was low to moderate in 2012 in the Baltic countries. Samples from several Latvian and Lithuanian fields were forwarded for testing. However, it was very difficult to make isolates from the samples. So in total only 24 isolates were tested from the two countries.

The sensitivity was quite in line with the sensitivity seen in both Denmark and Sweden (Table 5). Again a higher resistant factor was seen for prothioconazole compared with epoxiconazole. In both countries the sensitivity was slightly increased for both epoxiconazole and prothioconazole compared with data from 2011.

Table 5: Testing of isolates from Latvia and Lithuania.

		Epoxiconazole		Prothioconazole	
Lithuania	No. of isolates	EC_{50}	R factor	EC_{50}	R factor
1	3	0.21	11	12.6	84
2	2	0.16	8	8.5	57
3	2	0.07	4	5.1	34
4	2	0.12	6	11.5	77
5	2	0.1	5	1.3	9
Total	10	0.132	7	7.8	52
Latvia	No. of isolates	EC_{50}	R factor	EC_{50}	R factor
1	6	0.14	7	12.5	83
2	2	0.07	4	1.8	12
3	2	0.2	10	17.5	117
4	1	0.05	3	20.0	133
5	3	0.18	9	8.8	59
Total	14	0.128	6	12.1	81

Table 6: Summary of data Latvia and Lithuania from 2011 and 2012.

Lithuania	EC_{50} epoxiconazole	R factor	EC_{50} prothioconazole	R factor
2011 (14)	0.65	32	16	106
2012 (10)	0.13	7	7.8	52
Latvia	EC_{50} epoxiconazole	R factor	EC_{50} prothioconazole	R factor
2011 (5)	1.11	55	17.3	115
2012 (14)	0.128	6	12.1	81
Wild type IPO323	0.02		0.15	

Testing for sensitivity to difenoconazole, folpet and boscalid

36 isolates from Denmark and 37 isolates from Sweden were randomly chosen from the 2012 isolates for further testing. Testing for sensitivity to difenoconazole, boscalid and folpet were carried out in a similar way to the screening for sensitivity to epoxiconazole and prothioconazole. These products are relevant or will be relevant for Septoria control in the region.

The sensitivity to difenoconazole is very high and resistance factors are in line with epoxiconazole and lower than for prothioconazole (table 7 and figure 6). Boscalid had a very low resistant factor indicating that no shifting has taken place. A slightly higher EC_{50} value in Denmark could possibly reflect that the product is authorized here and has been for some years, whereas the product is not authorized in Sweden for cereals. Folpet is not authorized in the region yet. It is however noticeably that there is quite a big variation in sensitivity to this product and resistance factors are quite high, which is not easy to understand as the product has not been used. Similarly results for folpet were seen in a minor screening carried out in 2011. Due to this unexpected result for folpet 15 of the isolates were shared with Rothamsted Research Station. They could not confirm the Danish results, see table 7a, and therefore the Danish results are not to be trusted in this case. An investigation is going in order to be able to find what can have caused the differences obtained in the two labs (use of formulated product rather than technical product, less solubility of folpet in Danish agar, or other elements will be looked at).

Table 7: Screening data from sensitivity testing to difenoconazole, boscalid and folpet from randomly chosen isolates from 2012.

		Difenoconazole		Boscalid		Folpet	
	Number of isolates	EC_{50}	R factor	EC_{50}	R factor	EC_{50}	R factor
Sweden	37	0,036	14	0,28	1	10,6	52
Denmark	36	0,036	17	0,74	2	12,1	60
Reference isolates	IPO323	0,002		0,35		0,2	

Table 7a. Comparison of EC50 values between Rothamsted and AU for specific isolates tested for their sensitivity to folpet.

	folpet DK test	Chlorothalonil Uk test	folpet uk test
DK121101	64.9	0.24	1.77
DK121701	3.3	0.296	1.38
DK121402	8.6	0.167	0.874
DK122405	6.4	0.154	0.738
DK120502	10.8	0.196	1.32
DK120101	0.04	0.187	0.706
MS120304	0.12	0.515	1.8
MS120804	0.02	0.198	1.48
MS120904	3.4	0.195	1.22
MS120703	4.01	0.289	1.36
MS120601	0.26	0.179	1.79
MS121104	2.56	0.194	1.66
DK120407	33	0.256	1.21
DK122001	0.27	0.18	0.726
DK120708	3.6	0.198	1.17

R typing of isolates in NORBARAG trials

In order to be able to minimise and manage fungicide resistance, it is regarded to be valuable to obtain a better understanding of the changes in R-populations caused by different CYP51 mutations selected by triazoles and different control programmes.

A common trial plan for the NORBARAG region was carried out again in 2012 in order to study selection patterns from relevant control strategies, using products originating from different companies. The plan was similar to the plan carried out in 2011 (Table 8).

Table 8: Trial plan and sites with the 5 NORBARAG trials from 2012.

gs 33-37	gs 45-51
1. 0.5 Opus	0.5 Opus
2. 0.4 Proline	0.4 Proline
3. 0.4 Proline + 0.5 Sportak	0.4 Proline + 0.5 Sportak
4. 0.4 Proline	0.4 Armure
5. 0.63 Aviator	0.63 Aviator
6. 0.75 Bell	0.75 Bell
7. Untreated	Untreated

Trial No.:	Locality:	Variety:
12314-1	Denmark - Flakkebjerg	Hereford
12314-2	Denmark - Horsens LRØ	Hereford
12314-3	Sweden 1- Borrby Gärsnäs HUD 42	Boomer
12314-4	Sweden 2 - Mörarp Helsingborg HUD 43	Ellvis
12315-5	Lithuania 1	Zentos

Trial design and treatments

4 replicates and randomised plots. Spray as described in the trial plan. Control of weeds and pests and growth regulation is carried out as in the rest of the field. 5 trials were placed in the NORBARAG region. 20 leaves per plot with symptoms of *Septoria tritici* blotch were sampled at gs 75 and forwarded to the companies (**Bayer, BASF and Syngenta)** for characterisation of CYP51 mutations in the populations.

Results

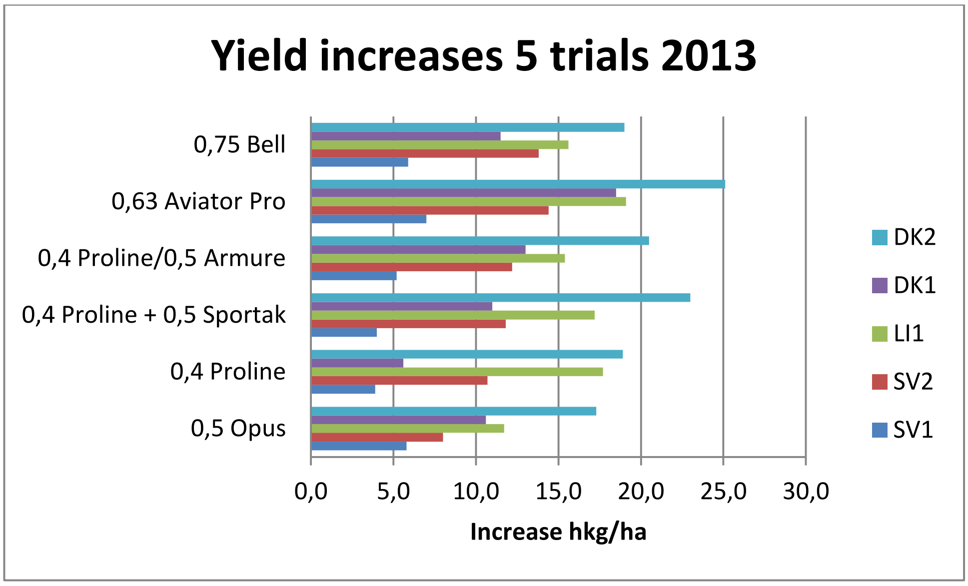
The 5 trials were carried out according to the trial plan. Variable amounts of Septoria developed in the trials. Level at gs 75 is shown in Table 9 and Figure 7 along with the yield responses.

Table 9: Main data from individual trials with control of Septoria (12314).

Yield and yield increases	Borrby SV1	Mörarp SV2	Lithuania 1	Flak DK1	LRØ DK2	Average	Rel. value
0.5 Opus	5.8	8.0	11.7	10.6	17.3	10.68	112
0.4 Proline	3.9	10.7	17.7	5.6	18.9	11.36	112
0.4 Proline + 0.5 Sportak	4.0	11.8	17.2	11.0	23.0	13.4	115
0.4 Proline/0.5 Armure	5.2	12.2	15.4	13.0	20.5	13.26	114
0.63 Aviator Pro	7.0	14.4	19.1	18.5	25.1	16.82	118
0.75 Bell	5.9	13.8	15.6	11.5	19.0	13.16	114
Untreated	113.6	88.5	75.0	97.3	86.6	92.2	100
LSD values	3.0	4.1		5.4	4.8		

% Septoria flag leaf gs 75-55	Borrby SV1	Mörarp SV2	Lithuania 1	Flak DK1	LRØ DK2	Average	% control
0.5 Opus	0.3	2.0	3.1	0.1	0.1	1.1	83.8
0.4 Proline	0.5	2.0	2.9	1.1	0.1	1.3	80.6
0.4 Proline + 0.5 Sportak	0.2	1.7	2.5	0.5	0.1	1.0	85.3
0.4 Proline/0.5 Armure	0.5	1.4	1.6	0.3	0.1	0.8	88.5
0.63 Aviator Pro	0.1	1.8	0.9	0.0	0.2	0.6	91.3
0.75 Bell	0.2	1.9	2.1	0.1	0.2	0.9	86.9
Untreated	4.8	3.3	7.6	10.0	9.0	6.9	

% Septoria 2nd leaf vs 75-77	Borrby SV1	Mörarp SV2	Lithuania 1	Flak DK1	LRØ DK2	Average	% control
0.5 Opus	5.0	8.3	10.7	1.6	8.3	6.8	81.8
0.4 Proline	8.5	6.3	6.8	12.5	9.5	8.7	77.7
0.4 Proline + 0.5 Sportak	4.3	5.5	3.2	2.3	7.0	4.5	88.6
0.4 Proline/0.5 Armure	6.8	8.5	4.9	2.3	6.5	5.8	85.1
0.63 Aviator Pro	3.0	4.3	1.6	0.1	2.3	2.3	94.2
0.75 Bell	3.5	7.5	7.1	0.6	11.5	6.0	84.5
Untreated	22.3	14.0	31.7	72.5	46.3	37.4	



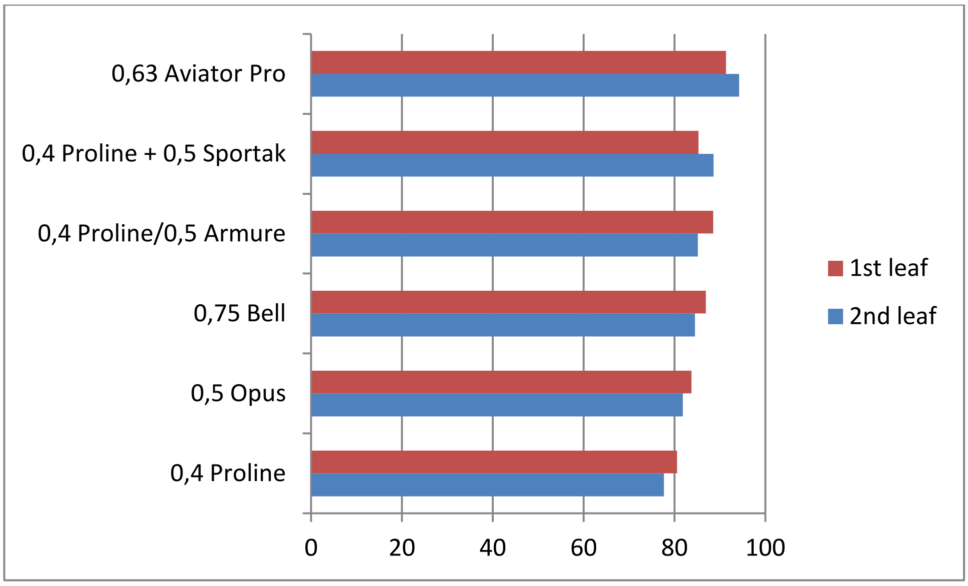


Figure 7: Summarising data NORBARAG trials from Denmark, Sweden and Lithuania.

Ring test

A sample from all 5 sites were forwarded to the 3 companies in order to see if the methods used gave comparable results. All tested mutations have been found in the samples originating from the 5 localities. The results are shown in Table 10. Comparing data from untreated samples analysed by all 3 companies falls with a few exceptions relatively close. Syngenta and BASF methods (pyrosequencing) are very similar and results match each other quite nicely. Bayer's method is different as they have tested mutations in single isolates. (6 per treatments). Bayer and Syngenta have assessed EC_{50} values for 3 triazoles using isolates from the 5 localities. In most cases Syngenta found higher values than Bayer.

Table 10: Comparing R typing of untreated plots (summer) from the 3 different companies. Frequency of occurrences of specific CYP51 mutations in the *Septoria tritici* populations.

	CYP 51 mutations							
Site	G134	A136	C136	G379	V381	T524	Company	
Flakkebjerg 1	**0%**	**7%**	**5%**	**63%**	**94%**	**0%**	Syngenta	Summer
Flakkebjerg 1	**0%**	**13%**	**0%**	**50%**	**86%**	**0%**	BASF	Summer
Flakkebjerg 1	**nt**	**4%**	**nt**	**38%**	**100%**	**0%**	Bayer	Summer
Horsens DK 2	**0%**	**8%**	**14%**	**51%**	**79%**	**0%**	Syngenta	Summer
Horsens DK 2	**0%**	**17%**	**0%**	**54%**	**85%**	**0%**	BASF	Summer
Horsens DK 2							Bayer	Summer
Borrby S 3	**6%**	**42%**	**3%**	**6%**	**62%**	**1%**	Syngenta	Summer
Borrby S 3	**0%**	**40%**	**0%**	**11%**	**77%**	**0%**	BASF	Summer
Borrby S 3							Bayer	
Mörarp S 4	**0%**	**3%**	**4%**	**16%**	**99%**	**0%**	Syngenta	Summer
Mörarp S 4	**0%**	**17%**	**0%**	**0%**	**93%**	**0%**	BASF	Summer
Mörarp S 4							Bayer	
Lithuania 5	**0%**	**2%**	**5%**	**40%**	**100%**	**0%**	Syngenta	Summer
Lithuania 5	**0%**	**0%**	**0%**	**37%**	**99%**	**0%**	BASF	Summer
Lithuania 5	nt	**29%**	nt	**29%**	**83%**	**16%**	Bayer	Summer

R typing following different treatments (Table 11):

BASF, Syngenta and Bayer, all find a big dominance of V381 and moderate amounts of G379 in Denmark and Lithuania, maybe less in Sweden. Furthermore there were minor amounts of T524 and A134, and low to moderate amounts of V136. Not many clear selection patterns could be seen in the material, although Sportak seems in 1-2 trials to select for A136. In one trial from Sweden Opus and Proline both select a lot for A135 and A136. Data are still missing from 2 trials which are under preparation from Bayer.

Table 11: Comparing R typing from different treatments in 3 sites 2012 (summer samples). Frequency of occurrences of specific CYP51 mutations in the *Septoria tritici* populations is shown.

		Often together		Rare	Often together		Often with V136A	
Borrby SV		D134G	V136A	V136C	A379G	I381V	S524T	Company
1	Opus	45%	78%	0%	11%	96%	0%	BASF
2	Proline	36%	76%	0%	4%	80%	0%	BASF
3	Proline + sportak	15%	84%	5%	0%	55%	0%	BASF
4	Proline + Armure	7%	34%	0%	15%	96%	0%	BASF
5	Aviator	0%	42%	0%	12%	85%	0%	BASF
6	Bell	7%	43%	0%	9%	89%	0%	BASF
7	Untreated	0%	21%	0%	12%	96%	0%	BASF
	Spring	0%	40%	0%	11%	77%	0%	BASF
LRØ DK		D134G	V136A	V136C	A379G	I381V	S524T	Company
1	Opus	0%	20%	3%	60%	87%	0%	BASF
2	Proline	0%	34%	0%	39%	74%	1%	BASF
3	Proline + sportak	0%	27%	3%	44%	80%	1%	BASF
4	Proline + Armure	0%	17%	0%	59%	90%	0%	BASF
5	Aviator	0%	11%	7%	47%	88%	0%	BASF
6	Bell	0%	22%	3%	44%	83%	0%	BASF
7	Untreated	0%	17%	0%	53%	85%	0%	BASF
	Spring	0%	13%	12%	53%	83%	0%	BASF
Lithuania		D134G	V136A	V136C	A379G	I381V	S524T	Company
1	0.5 Opus	0%	1%	5%	51%	100%	0%	Syngenta
2	0.4 Proline	0%	2%	2%	20%	100%	0%	Syngenta
3	0.4 Proline + 0.5 Sportak	0%	4%	1%	19%	100%	0%	Syngenta
4	0.4 Proline/0.5 Armure	0%	1%	3%	44%	100%	0%	Syngenta
5	0.63 Aviator Pro	0%	1%	3%	37%	100%	0%	Syngenta
6	0.75 Bell	0%	1%	6%	36%	100%	1%	Syngenta
7	Untreated	0%	1%	3%	35%	100%	1%	Syngenta
	Spring	0%	2%	2%	38%	98%	0%	Syngenta