Aarhus University
Faculty of Agricultural Sciences
Department of Integrated Pest Management

Data from monitoring for fungicide resistance in cereals 2008

Drechslera teres isolates from Sweden and Denmark

Septoria tritici isolates from Denmark and Sweden

Drechslera tritici-repentis isolates from Denmark

Blumeria graminis f. Sp. hordei from Denmark

Created by: Lise Nistrup Jørgensen
Aarhus University
Faculty of Agricultural Sciences
Department of Integrated Pest Management
Research Centre Flakkebjerg
DK-4200 Slagelse

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Trial leader: Lise Nistrup Jørgensen

Research assistants: Lis Henriksen/Jette Lilholt/Lone Fink/Helene Saltoft

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1. SUMMARY

Septoria tritici

The level of Septoria leaf blotch was extremely low in 2008 in both Denmark and Sweden and therefore it was not possible to measure the products' field performances against septoria leaf blotch to any major extent. In Denmark mainly epoxiconazole and prothioconazole are used for control of septoria whereas in Sweden mainly prothioconazole is used.

Denmark

A total of 88 isolates of *Septoria tritici* from 15 leaf samples picked at GS 73-77 in Denmark in 2008 were isolated and tested for their sensitivity to epoxiconazole. The isolates originated from 7 different localities. The number of isolates per sample varied from 1 to 11 isolates. EC_{50} values varied between 0.02 and 0.34 ppm. The average value was 0.17 ppm. No significant differences in EC_{50} values were found between different localities. The EC_{50} values for epoxiconazole values were lower in 2008 compared with the data from 2006 and 2007. No isolates with EC_{50} values higher than 1.0 ppm were found in 2008. Measured in relation to the reference isolates, which had an EC_{50} value of 0.04 ppm, the average resistance factor to epoxiconazole is calculated to be around 4.

Sweden

A total of 257 isolates of *Septoria tritic*i from 33 leaf samples picked at GS 71-77 in Sweden in 2008 were tested for sensitivity to epoxiconazole. Further 56 isolates were also screened for sensitivity to prothioconazole. The number of isolates from the individual fields varied from 2 to13, with an average of 8 isolates per locality. EC_{50} values to epoxiconazole varied all over between 0.01 and 3.8 ppm. The average value measured for the different localities varied between 0.21 and 0.97 ppm. The overall average is 0.38 ppm. Significant differences in EC_{50} values were found between different localities. When the results from 2008 are compared with those from 2007, the data show a high degree of similarity. Ten isolates had EC_{50} values higher than 1.0 ppm. Measured in relation to the reference isolates, which had an EC_{50} value around 0.04 ppm, the average resistance factor is calculated to be around 10.

With respect to prothioconazole the average EC_{50} value was 1.5 ppm varying between 0.14 and 4.2 ppm. The reference isolates had EC_{50} values of 0.12-0.14 ppm. Although the EC_{50} values were higher for prothioconazole than for epoxiconazole the data similarly gave an average resistance factor of approximately 10.

Drechslera teres

Denmark

The attack of net blotch was low in 2008 due to drought and therefore it was difficult to get many samples with attack. Out of 38 collected samples only 20 had sufficient net blotch to analyse for

F129L mutations. 11 of those 20 samples had F129L (55%) to a varying degree. 1 sample < 10%; 6 samples: 10-49%; 4 samples > 50%. The analysis for F129L was carried out by BASF.
No direct impact from F129L on field performance using strobilurins was seen in 2008, but results from a semi-field trial with approximately 50% of the isolates having F129L showed a clear ranking of the performance from strobilurins: pyraclostrobin > picoxystrobin> azoxystrobin.

In a bioassay 161 isolates were tested for sensitivity to the DMI fungicides imazalil, prothioconazole and propiconazole. Also a smaller number were tested for sensitivity to azoxystrobin. The average EC_{50} values for the four products were 6.88 ppm, 14.8 ppm, 25.2 ppm and 1.7 ppm respectively. A significant but not very clear correlation between EC_{50} values for the 3 DMI fungicides were measured. The best correlation was found between imazalil and propiconazole with an R^2= 0.49. The variation between isolate sensitivity to DMIs is significant and indicates that shifting in sensitivity could have taken place. Significant differences in EC_{50} values were measured between some of the localities. Unfortunately, a lack of very old reference isolates does not make it possible to calculate reliable resistance factors.

Sweden

Also in Sweden it was very difficult to find good and clear symptoms in 2008 due to drought. 13 samples were analysed for the strobilurin mutation F129L in *D. teres*, but net blotch was only found in 7 samples. Out of the tested samples only 2 samples carried the F129L mutation.

83 isolates from 12 localities were tested in a bioassay for sensitivity to imazalil, prothioconazole and propiconazole. A smaller number of isolates (16) were tested for sensitivity to azoxystrobin. The average EC_{50} values for the four products were 7.7 ppm, 21.23 ppm, 31.07 ppm and 0.42 ppm respectively. No significant differences were measured between localities. As for the Danish isolates, the variation between isolate sensitivity to DMIs was seen to be significant, which indicates that shifting in sensitivity could have taken place. Unfortunately, a lack of old reference isolates does not make it possible to calculate reliable resistance factors.

Drechslera tritici–repentis

I 2008 the level of tan spot was relatively low due to dry conditions and the disease did not develop to any significant level in most fields.

Denmark

In 2008 leaf samples from 9 localities were collected at GS 65-75 and tested for strobilurin resistance using PCR-based methods carried out by BASF. The samples were analysed for 3 mutations known to be linked to strobilurin resistance (F129L, R137R and G143A). All 3 mutations are common in the Danish population with an average occurrence of 48% G142A, 41% F129L and 5% G137R. All 3 mutations were in several cases present in the same field although some samples showed dominance of one or the other. Two localities were sampled both spring and summer and results did not show a great degree of consistency between occurrences of mutations. Summer data from different fungicide treatments showed major variation within a field and only slight changes during the season following treatments with strobilurins compared with other treatments.

Blumeria graminis f.sp. hordei

A limited number of samples from Denmark (11) were analysed in 2008 for occurrence of G143A mutations in the powdery mildew population in barley. Four of the samples showed between 10 and 41% occurrence of G143A. A clear drop in field performance from strobilurins was seen in the case in which 41% of the population had developed resistance.

2. AIM

- To test the sensitivity of *D. teres* to strobilurins and triazoles as part of a monitoring programme for diseases in Denmark and Sweden.
- To test the sensitivity of *Septoria tritici* isolates from Denmark and Sweden to epoxiconazole as part of a monitoring.
- To test the sensitivity of *Septoria tritici* isolates from Sweden to prothioconazole in a none specified number of isolates.
- To test sensitivity to strobilurins in minor populations of *Blumeria graminis f.sp hordei* and *Drechslera tritici-repentis*.
- The overall aim is to be able to adjust recommendation in accordance with the development of fungicide resistance in wheat.

3. METHODS AND MATERIALS

Drechslera tritici repentis

20-30 leaves were sampled around GS 75 from 9 Danish localities in 2008. A few localities were sampled twice, before and after fungicide treatments. Samples were taken from fungicide trials.
On arrival the samples were dried. Leaves were forwarded to BASF for assessments of strobilurin mutations using PCR-based method.

In two fungicide trials leaves were sampled around GS 75 from all treatments. Two replicates were analysed for mutation, again using PCR based–methods.

Septoria tritici

Around GS 71-75 leaf samples with 30-40 leaves were picked and sent to Flakkebjerg. A total of 15 samples were investigated from Demark and 33 samples from Sweden. 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 ppm streptomycin and 0.01 ppm rifamplicine for propagation.

***In vitro* test**

Sensitivity towards epoxiconazole and prothioconazole was estimated on the basis of approximately 10 pure, cultured isolates per leaf sample aiming at 1 isolate per leaf. A spore suspension containing 5-10 x 10^4 spores/mL of each fungal isolate (two replicates) was produced from 5-day-old potato dextrose agar-grown blastospores and transferred to a microtitre plate (CM-LAB) with 7 different concentrations of epoxiconazole a.i. (0.01; 0.03; 0.1; 0.3; 1 and 10 ppm/L) mixed with potato dextrose agar (DIFCO). The reference isolates S27 (WT) and RL2 (D. M. Hollomon, Long Ashton Research Station, UK), with known epoxiconazole EC_{50} values of approximately 0.01 ppm were also included on one plate, in order to ensure correct conditions. The micro plates were incubated at 22°C in the dark for 6 days, after which per cent growth was visually assessed relative to control, by designating into 5% intervals. EC_{50} values were estimated by means of Graph prism software.

Drechslera teres

Leaf samples were collected from trial sites. Samples were taken from both untreated and fungicide-treated plots. 40 leaves per samples were picked. On arrival the samples were dried. Half of the sample was sent to BASF for screening for F129L mutations. The second part of the sample was tested for sensitivity to different triazoles.

For this purpose the samples were rehydrated and placed in boxes with wet filter paper. After 24 hours, the leaves were checked for *D. teres* mycelial or spore growth. If no growth was observed, the leaves were incubated for a further 24h. In general, mycelium and spores were observed after a total incubation period of 48h.

When growth had been detected, mycelium or spores from healthiest lesions were transferred, using a sterile needle, to grass agar (65 G grass “pellets” +20 g Bacto agar per litre). It has been aimed at getting ten isolates per locality using 1 isolate per leaf. The plates were incubated for 7 days at 22°C under conditions of 12 h black light, 12 h dark.

After 7 days, the colonies were checked for contamination, and if none was observed, the colonies were sub-cultured onto 2 plates with grass agar. After 14 days’ incubation (22°C, 12h black light/12h dark), the colonies were again checked for contamination and discarded if contamination was observed. 1 plate was used for the resistant testing and one plate was stored as a reserve.

After the two weeks’ incubation, mycelium and spores (if present) were harvested into sterile deionised water and blended using a homogeniser.

***In vitro* test**
Microtitre plates were prepared with alkyl ester (AE) agar containing different concentrations of fungicides:

- Prothioconazole: 0.01 ppm, 0.1 ppm, 1.0 ppm, 5 ppm, 10 ppm, 25 ppm and 50 ppm.
- Imazalil: 0.01 ppm, 0.1 ppm, 1.0 ppm, 5 ppm, 10 ppm, 25 ppm and 50 ppm.
- Propiconazole: 0.01 ppm, 0.1 ppm, 1.0 ppm, 5 ppm, 10 ppm, 25 ppm and 50 ppm.
- Azoxystrobin: 0.01 ppm, 0.1 ppm, 1.0 ppm, 5 ppm, 10 ppm, 25 ppm and 50 ppm.

The plates were prepared the same day they were inoculated. The microtitre plates were inoculated with spore suspension and plates were inoculated with 100,000 spores per ml. The plates were incubated at 22°C under dark conditions for 7 days.
Two reference isolates borrowed from Syngenta, Stein were included in the test. (DK 2/2 from 1997 and F2588 from 1988). Unfortunately, no reference isolates going back to prior DMI use were available.

After 7 days, the micro plates were assessed, where percent growth was visually assessed relative to control, by designating into 5% intervals. EC_{50} values were estimated by means of Graph prism software.

4. RESULTS

Septoria tritici

<u>Denmark</u>
The disease level of Septoria leaf blotch in 2008 was very low due to drought and therefore only few samples were sampled as symptoms around GS 75 were very rare.

From Denmark only 88 isolates originating from 15 leaf samples were tested and gave EC_{50} values between 0.02 and 0.34 ppm. The average value was 0.17ppm, which was 0.5 ?? lower than 2007 (0.77 ppm in 2007 and 0.57 ppm in 2006). No significant differences in EC_{50} values were found between different localities in 2008 (Figure 1). The average EC_{50} values for the samples varied from 0.07 to 0.23 ppm. No samples had EC_{50} values which were higher than 1.0 ppm. Comparing the sensitivity of the tested isolates with the sensitivity in the reference isolates (EC_{50}= 0.04 ppm) it is, however, still clear that a significant change in sensitivity has taken place since the start of the triazole era.

Figure 2 shows the frequency of isolates belonging to the different sensitivity groups. Generally there seems to have been an increase in sensitivity compared with 2007 where a higher proportion of isolates with EC_{50} values higher than 1.0 ppm were found. Data from 2008 seem to be very similar to data from 2005 (47 isolates). That year the EC_{50} was on average 0.12 ppm varying from 0.02 to 0.81 ppm (Figure 2).

As the disease level of Septoria leaf blotch in 2008 was very low it was not possible to check the field performance from epoxiconazole in 2008.

Table 1. List of average EC_{50} values for epoxiconazole from different localities in Denmark.

Locality	Loc. no.	No. of isolates	EC_{50} for epoxiconazole
VestJylland	1	1	0.1817
Flakkebjerg, Baltimor	3	9	0.2329
Kolding	4	1	0.07981
Fyn	7	11	0.1683
Landbo midtøst	9	1	0.1697
LRØ Horsens	11	1	0.1393
Flakkebjerg, Baltimor	12	8	0.1909
Flakkebjerg, Baltimor	13	10	0.1802
Flakkebjerg, Baltimor	14	9	0.1582
Flakkebjerg, Ritmo	16	10	0.1858
Grindsted	18	5	0.1316
Grindsted	20	1	0.126
Flakkebjerg, Robigus	22	7	0.1741
Flakkebjerg, Baltimor	23	9	0.1658
Flakkebjerg, Ritmo	24	5	0.1514
	ref	8	0.04379

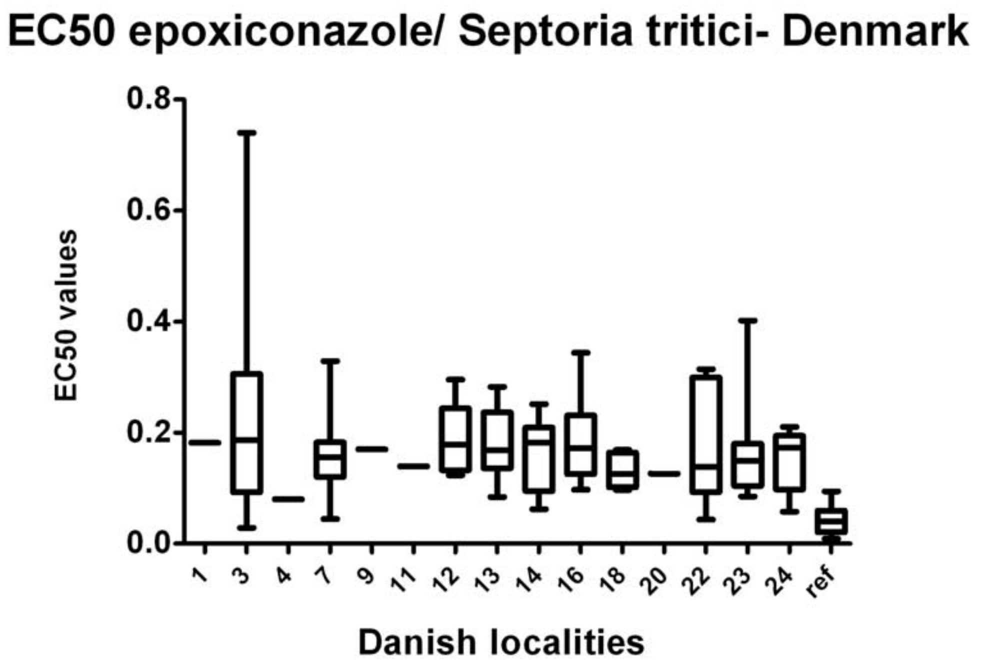


Figure 1. EC_{50} for epoxiconazole for Danish localities in 2008. The distribution of EC_{50} values is illustrated with box and whisker plots. No significant differences between localities were measured.

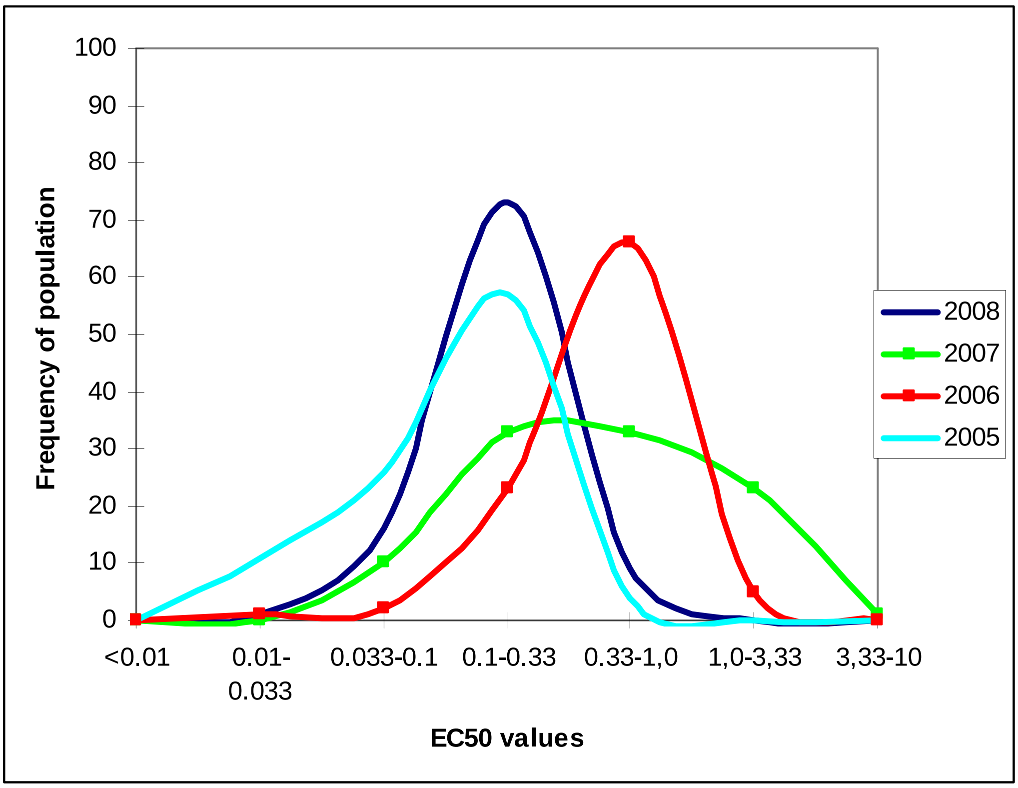


Figure 2. Distribution of sensitivity to epoxiconazole analysing Danish isolates from 4 years.

<u>Sweden</u>

A total of 257 isolates from 33 leaf samples (mainly flag leaves) were picked at GS 75 from the middle and south part of Sweden in 2008. Most samples came from trials which were treated with different dosages and fungicide strategies using mainly prothioconazole. The fungi were isolated and tested for their sensitivity to epoxiconazole and prothioconazole. The number from the individual field varied from 3 to 13, with an average of 8 isolates per locality being tested for sensitivity to epoxiconazole. The bioassays screened the isolate sensitivity on 0.01, 0.033, 0.1, 0.33, 1.0, 3.3 and 10 ppm of the active ingredients. Results are shown in Table 2.

Table 2. List of average EC_{50} values for epoxiconazole from different localities in Sweden.

Locality	**Locality no.**	**No of isolates tested for epox.**	**EC_{50} values for epoxiconazole**	**EC_{50} values for prothioconanzole**
Vinberge Fågelstad	1	10	0.25	1.01
Hyttringe Fornåsa	2	8	0.71	0.30
Lårstad Motala	3	9	0.32	1.81
Kolback Fornåsa	4	6	0.38	0.65
Hallebergega Klockrike	5	4	0.10	0.58
Bobjerg Fornåsa	6	7	0.33	0.60
Åaby Vaderstad	7	9	0.55	0.91
Marstad Skånningen	8	8	0.23	0.55
Forsa Mjølby	9	5	0.38	1.11
Angelholm vaxtskyddscentralen Alnarp	10	5	0.41	1.52
Angelholm vaxtskyddsc. Alnarp	11	11	0.44	1.12
Kæmpetorp	12	12	0.32	1.25
Ingarud-Mariastab	13a	2	0.38	1.53
Torestorp- skovde	13b	8	0.55	1.53
Håberg græstorp	14	10	0.60	1.53
Skøjteby Lidkoping	15	8	0.27	-
Laggaea gården græstorp	16	10	0.58	2.41
Torpa græstorp	17	11	0.43	1.81
Håberg græstorp	18	12	0.55	1.01
L7-101 Vauåkra	19	5	0.33	3.95
L15-1050 stuppåkra	20	7	0.23	1.62
Lønnstorp Lomma	21	3	0.30	0.75
L15-1050 Trelleborg	22	7	0.98	2.25
L15-105 Wilhelmfalt angelholm	23	10	0.29	3.26
"93" near Vasterås	24	9	0.26	1.92
L7-101 Angelholm	25	8	0.21	1.02
L7-101 Angelholm	26	13	0.25	3.02
Peder Waen	27	12	0.24	1.08
Hagby Øland	28	10	0.21	2.35
Karlsborg, Kalmer ubeh,	29	5	0.59	1.74
Karlsborg, Kalmer beh,	30	7	0.23	1.72
Hagby Øland Borgholm	31	2	0.24	3.22
Rinkabt;Kalmer	32	4	0.29	0.22
Average EC_{50} values		257	0.38	1.47 (54 isolates)
Reference isolates		2	0.044	0.13
Average resistance factor			8.8	11.3

EC_{50} values varied between 0.01 and 3.8 ppm. The average value measured for the different localities varied between 0.21 and 0.97 ppm (Table 2 and Figure 3). The overall average was 0.38 ppm, which is very much in line with 0.32 ppm measured in 2007. Significant differences in EC_{50} values were found between different localities. The average of the reference isolates gave EC_{50} values of 0.043 ppm. Only 7 localities (a total of 10 isolates) had EC_{50} values higher than 1.0 ppm. When the data from 2007 are compared with the results from 2008, the results are generally very similar indicating the same level of sensitivity. The EC_{50} values from 2008 in Sweden were slightly higher than in Denmark.

A total of 54 isolates were tested for their sensitivity to prothioconazole. The aim was to test 2 isolates per locality. The bioassays screened the isolates sensitivity on 0.01, 0.033, 0.1, 0.33, 1.0, 3.3 and 10 ppm of the active ingredient. With respect to prothioconazole the average EC_{50} values were 1.5 ppm, with a range of 0.14-4.2 ppm. Most localities had isolates with sensitivity between 1 and 2 ppm. The reference isolates had EC_{50} values of 0.12-0.14 ppm. Although the EC_{50} values were higher for prothioconazole than for epoxiconazole, the data similarly gave an average resistance factor of approximately 10.

In Sweden epoxiconazole is not available so control of septoria is mainly done using prothioconazole. The efficacy in the trials have generally been acceptable using prothioconazole, but as the disease level of Septoria leaf blotch in 2008 was very low, it was not possible to check the field performance from prothioconazole in 2008

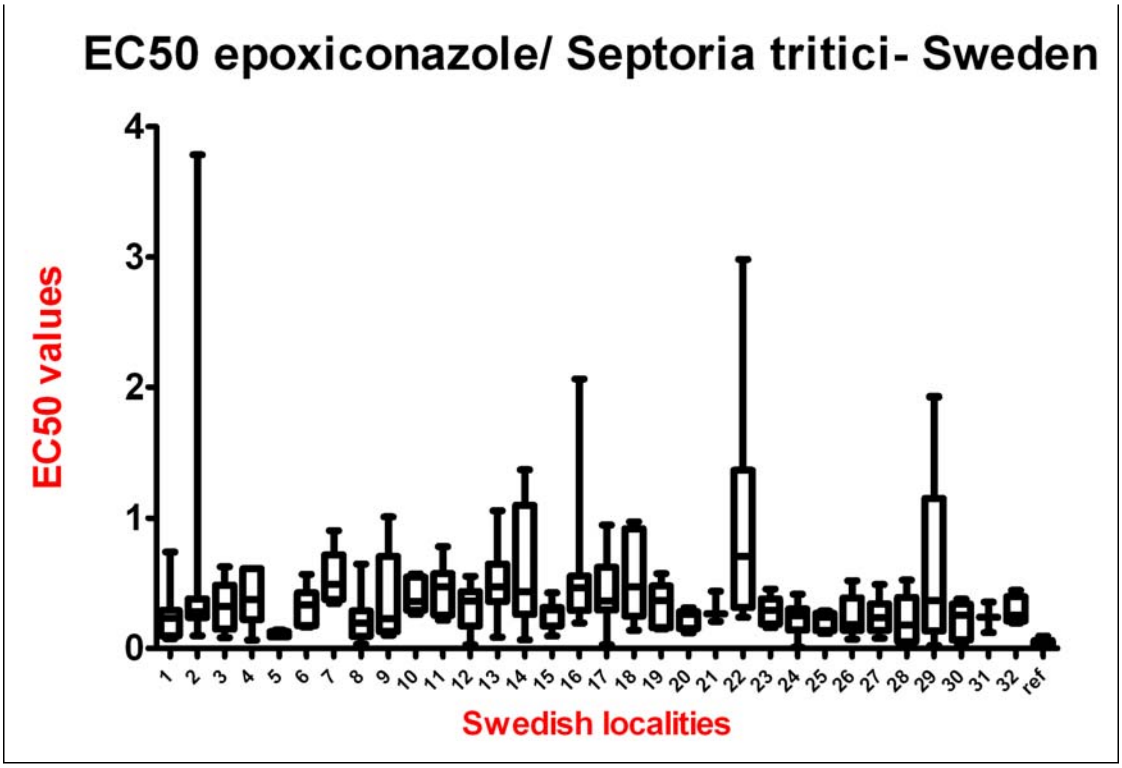


Figure 3. EC_{50} for epoxiconazole for Swedish localities in 2008. The distribution of EC_{50} values is illustrated with box and whisker plots. Significant differences between localities were measured.

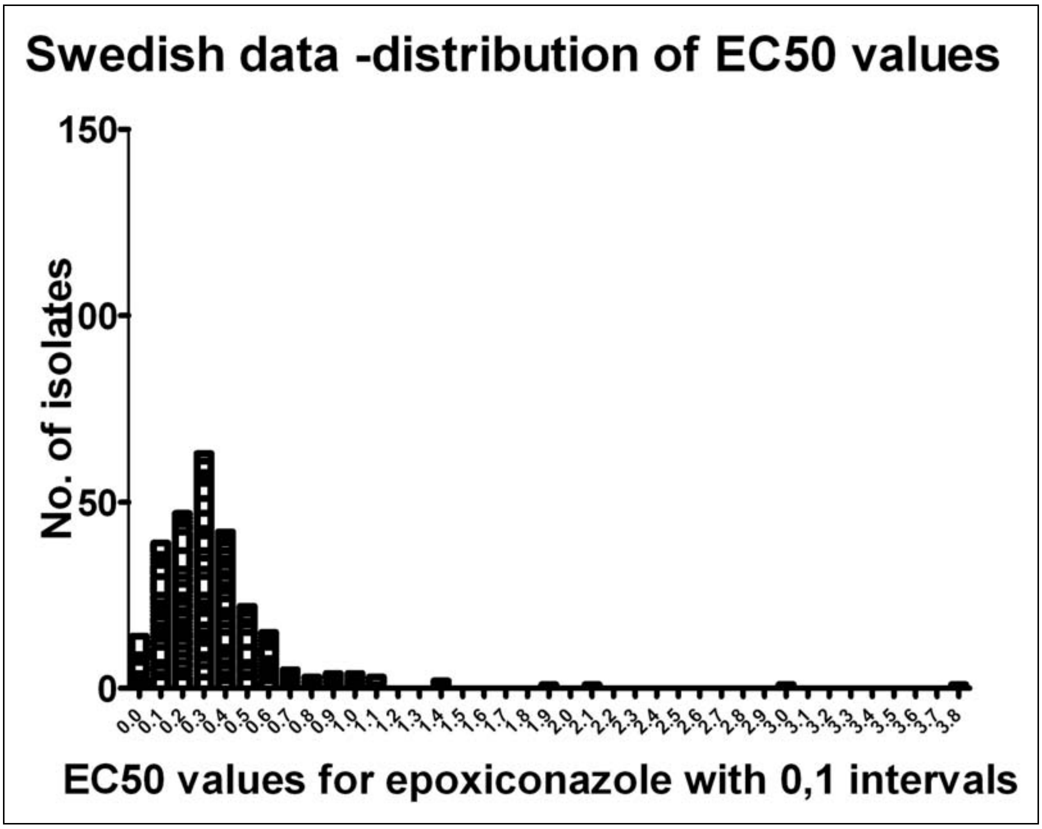


Figure 4. Distribution of sensitivity to epoxiconazole Analyzing 259 isolates from 33 localities in Sweden in 2008.

Drechslera teres

Strobilurin resistance in the *D. teres* population.
The attack of net blotch was low in 2008 due to drought and therefore it was difficult to get many samples with attack. Out of 38 collected samples only 20 had sufficient net blotch to analyse for F129L mutations. 11 of those 20 samples had F129L (55%) to a varying degree. 1 sample < 10% 6 samples: 10-49%; 4 samples > 50%. The analysis was carried out by BASF.
No direct impact on field performance was seen in 2008, but results from France and the UK indicate that the less potent strobilurins in particular give lower levels of control once the F129L population becomes widespread. Although cross resistance between strobilurins is well known results show that e.g. pyraclostrobin is less affected by the F129 L mutation than e.g. azoxystrobin.

Table 3. List of samples with net blotch collected in 2008 and analysed for stobilurin resistance using the frequency of F129L as indicator. Samples are from summer and autumn samples.

Locality	Region	% F129L
Auming	Rønde	0
Ørre	Aulum	0
Nautrup	Aulum	90
Hornslet	Rønde	17
Sdr. Bjerge	Slagelse	8
Hornslet	Rønde	18
Allingåbro	Rønde	0
Randers	Randers	72
Høstrup	Holstebro	0
Smidie	Hadsten	12
Sønderholm	Hadsten	13
Toreby	Nykøbing F.	0
Byskov	Nykøbing F.	31
Ø. Toreby	Nykøbing F.	0
Skanderborg	Århus	0
Bjærup	Slagelse	0
Erdrup	Slagelse	64
Sdr. Bjerge	Slagelse	0
Ottestrup	Slagelse	49
V. Broby	Sorø	50

The efficacy from different strobilurins was tested in a semi-field trial carried out at Flakkebjerg, where a bulk population of *D.teres* with approximately 50% of the isolates carrying the mutation gave a clear ranking in efficacy between the tested products showing Comet > Aproach> Amistar (Figure 5). Proline, which is a triazole, gave control similar to Aproach in the trial.

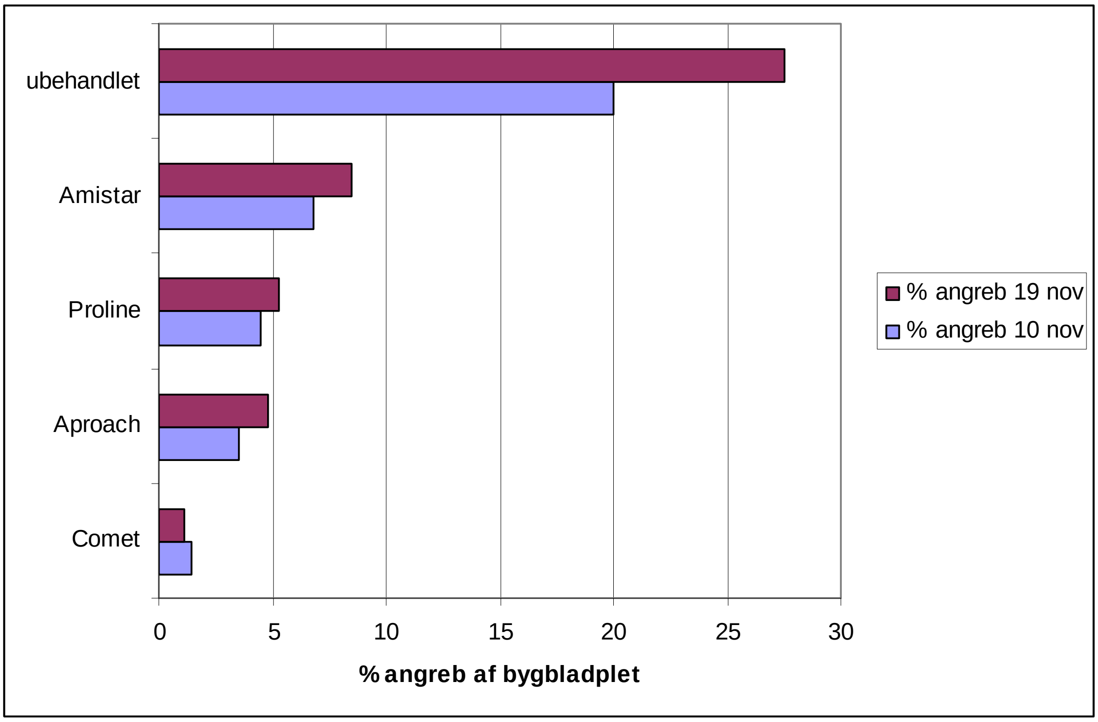


Figure 5. Effectiveness from 4 fungicides tested at half rate for control of net blotch inoculated under semifield conditions in the autumn. The plants were inoculated with a spore suspension of isolates with and without F129L. The trial was inoculated on 24 October and sprayed on 29 October.

Strobilurin resistance in Sweden

Samples of net blotch were also collected from Sweden.

Again it was very difficult to find good and clear symptoms due to drought. 13 samples were analysed but net blotch were only found in 7 samples. Out of the tested samples only 2 samples carried the F129L mutations.

Table 4. List of samples with net blotch collected in 2008 in Sweden and analysed for stobilurin resistance using the frequency of F129L as indicator. Samples are from summer samples.

Locality	Region	% F129L
Tomelilla-Ulstorp	**Skåne**	**20**
Romelilla-Hedeberga	**Skåne**	**35**
Kristiansstad	**Skåne**	**0**
Brakne-Hoby	**Skåne**	**0**
Orsa	**Uppsala**	**0**
Gustafs	**Uppsala**	**0**
Røloppe	**Uppsala**	**0**
Rasbo	**Uppsala**	**0**

Triazole sensitivity

In the bioassay 161 Danish isolates were tested for sensitivity to imazalil, prothioconazole and propiconazole. Only 18 isolates were tested for sensitivity to azoxystrobin. All products were tested at 0.01; 0.1; 1.0; 5.0; 10; 25 and 50 ppm. The average EC_{50} values for the four products varied considerably.

The EC_{50} values for imazalil varied between 0.32 ppm and 38.6 ppm with an average of 6.88 ppm. For prothioconazole the same values varied between 2.2 and 56.8 ppm with an average of 14.8 ppm. For propiconazole the values tended to be higher than for the two other products with average EC_{50} values of 25.2 and a variation between 2.5 and 59.7 ppm (Table 6 and Figure 7).

An analysis of the variation between localities showed significant differences between several of the localities. Correlations between EC_{50} values for the 3 DMI products imazalil, prothioconazole and propiconazole showed the expected trend, but the correlation coefficient was relatively poor (Figure 6). The best correlation was found between imazalil and propiconazole with a R^2= 0.49.

One of the two reference isolates originate from Syngenta (F2588) and the other one is a relativy old one from DK (1997), but neither is going back very long. The average EC_{50} values for the two isolates were for imazalil 1.76, prothioconazole 1.2 and propiconazole 25.7 respectively (Table 5). The major variation in sensitivity to the 3 DMI products also led to large variation in the average resistance factors, which was calculated to 4.04, 13.0 and 1.0 for imazalil, prothioconazole and propiconazole respectively. The lack of very old isolates, which should reflect the sensitivity before triazoles were initially used, makes it very difficult to calculate reliable resistance factors.

Table 5. Reference isolates of *D. teres*.

	F2588 from 1988	DK2/2 from 1997	Average
Imazalil	2.28	1.24	1.76
Prothioconazole	0.37	1.98	1.2
Propiconazole	36.40	15.0	25.7

Sweden

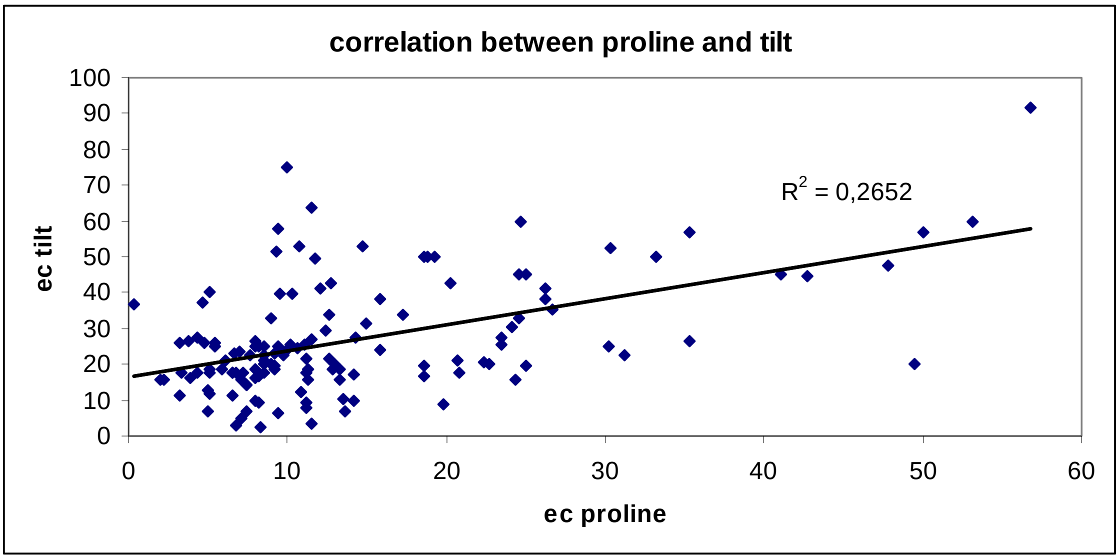
83 isolates from 12 localities were tested in a bioassay for sensitivity to imazalil, prothioconazole and propiconazole. And a smaller number (16) were tested for sensitivity to azoxystrobin. All products were tested at 0.01; 0.1; 1.0; 5.0; 10; 25 and 50 ppm. The average EC_{50} values for the four products varied considerably, but no significant differences were measured between localities. The ranking between the 3 products found in Denmark was similarly seen for the Swedish isolates (Table 6 and Figure 8).

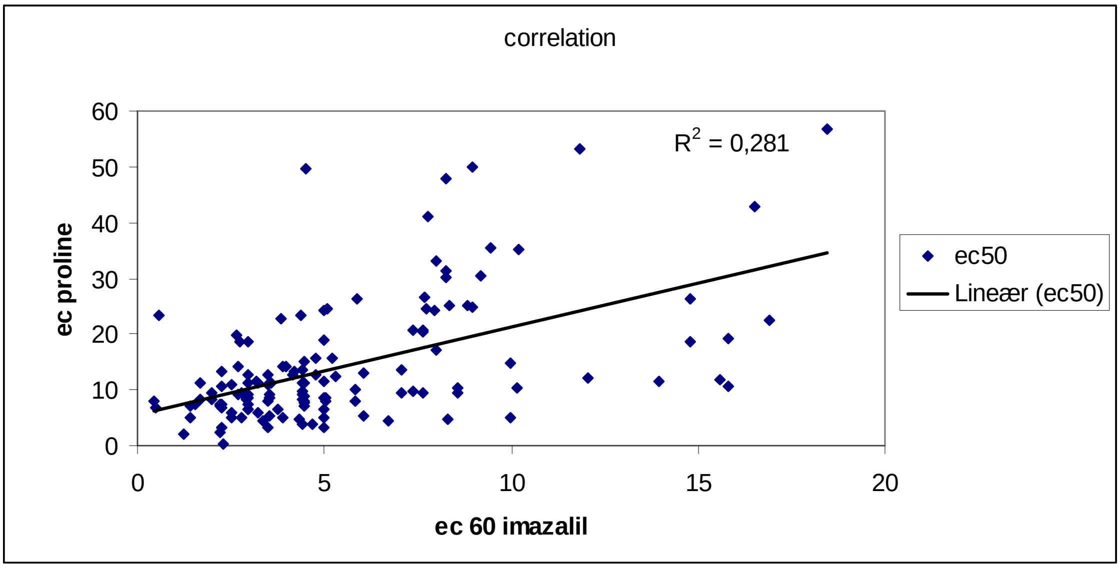
The EC_{50} values for imazalil varied between 0.32 ppm and 23.2 ppm with an average of 7.7 ppm. For prothioconazole the same values varied between 0.37 and 59.75 ppm with an average of 21.23 ppm. For propiconazole the values tended to be higher than for the two other products with average EC_{50} values of 31.07 and a variation between 0.9 and 52.2 ppm.

Using the same reference isolates as in the Danish screening (table 5) the average resistance factors can be calculated to 5, 19 and 1 respectively for imazalil, prothioconazole and propiconazole. This indicates, however, that the reference isolates most likely are from post propiconazole time, as a shift in sensitivity to this product already has taken place in the two reference isolates.

Table 6. List of average EC_{50} values for imazalil, prothioconazole, propicoanzole and azoxystrobin in Denmark.

Locality	**Locality no.**	**No. of isolates**	**EC_{50} values for imazalil**	**EC_{50} values for prothio-conazole**	**EC_{50} values for propico-nazole**	**EC_{50} values for azoxy-strobin**
Flakkebjerg	1	10	3.39	10.11	16.13	0.076
Auning, Rønde	2	11	2.80	9.05	15.10	0.56
Ørre Aulum	3	10	4.58	15.68	21.98	0.109
Nautrup, Aulum	4	10	4.12	9.88	22.17	0.031
Hornslet, Rønde	5	10	6.98	19.40	24.04	0.102
Sdr. Bjerge, Slagelse	6	10	3.93	10.21	17.04	0.16
Hornslet, Rønde	8	10	4.05	15.33	30.63	0.267
Allingåbro, Rønde	9	7	2.99	9.73	18.63	0.10
Randers,	16	4	8.22	33.59	49.14	-
Toreby	17	4	6.87	21.57	23.59	2.65
Holeby	19	1	8.24	31.21	22.42	10.6
Høstrup, Holstebro	29	8	18.33	20.23	33.01	0.076
Hobro	30	1	8.33	25.09	19.40	0.316
Smidie, Hadsten	33	2	9.99	14.76	40.92	
Klarup, Hadsten	34	5	10.34	14.11	38.63	11.98
Søndermark, Hadsten	35	3	4.80	4.32	20.69	
Barsbøl, Hadsten	36	1	16.9	22.38	20.83	
Virket, Nykøbing F	37	1	31.32	-	10.99	
Toreby Nykøbing F	46	7	5.32	10.15	23.43	
Byskov, Nykøbing F	47	4	2.81	4.63	14.55	
Øster Torby, Nykøbing F	48	5	10.54	19.39	50.78	
Bjærup, Slagelse	50	4	10.49	54.81	36.39	
Erdrup, Slagelse	51	7	5.03	9.85	28.03	
Sdr. Bjerge, Slagelse	52	4	12.6	28.87	42.83	
Ottestrup, Slagelse	53	3	10.65	25.87	47.21	
V.Broby	54	5	8.56	32.64	39.58	
Karup, Jylland (Kloster)	55	8	4.73	10.00	22.33	
Karup, Jylland (Kloster)	56	6	11.26	13.24	40.19	
Average EC_{50} values			6.88	14.76	25.23	1.71 (18)
Reference isolates			1.76	1.2	25.0	
Average resistance factor			3.9	12.3	1.0	





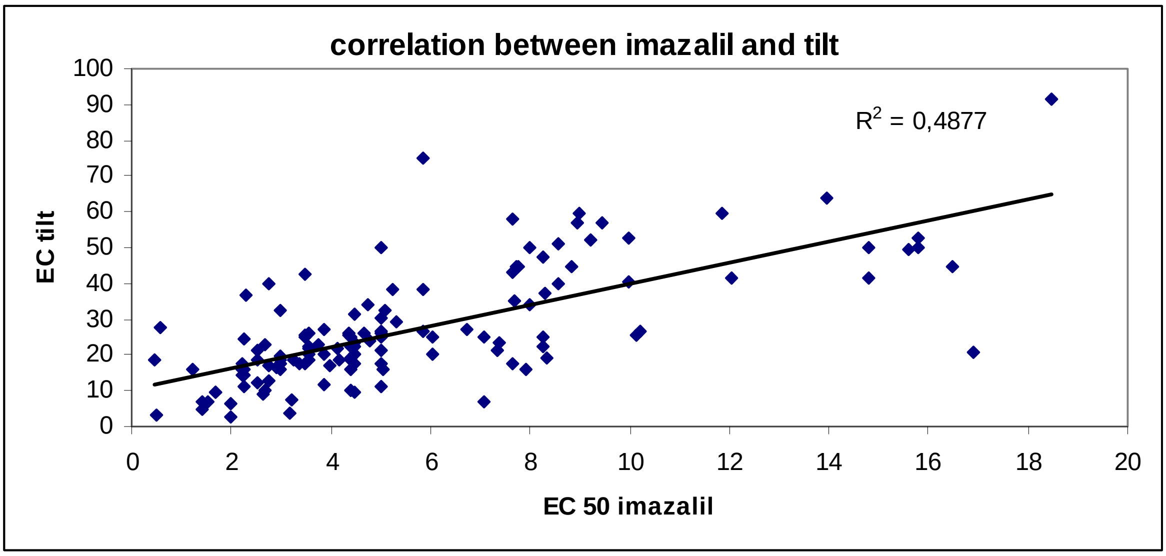


Figure 6. Correlations between EC_{50} values for *D. teres* for 3 different DMI fungicides.

Table 7. List of average EC_{50} values for imazalil, prothioconazole, propiconazole and azoxystrobin in Sweden.

Locality	**Locality no.**	**No. of isolates**	**EC_{50} values for imazalil**	**EC_{50} values for prothioco-nazole**	**EC_{50} values for propicona-zole**	**EC_{50} values for azoxystro-bin**
Tormellila, Ullstorp	110	8	9.4	27.7	42.06	0.61
Tormelilla, Hedeberg	111	7	5.66	17.59	2.9.92	0.21
Kristiansstad	112	6	7.04	18.68	36.75	-
Brakne-Hoby	113	6	6.01	29.41	-31.53	0.21
As Katrineholm S	131	9	7.65	38.25	33.8	0.88
Flistad, Linkøping S	132	8	9.19	33.3	45.46	0.37
Orsa, Upsalla	140	3	7.03	7.2	4.40	
St. Skedevi, Upplsala	141	6	6.34	39.24	37.89	
Gustafs, Uppsala	142	7	7.22	15.833	27.81	
Röloppe, Uppsala	143	10	10.15	15.13	25.15	
Rasbo, Uppsala	144	9	6.12	15.48	26.36	
Sollebrun, Skara	145	4	8.83	20.78	38.63	
Total number		83	83	83	66	16
Average EC50 values			7.69	21.23	31.25	0.425
Reference isolates			1.7	1.2	15.0	
Average resistance factor						

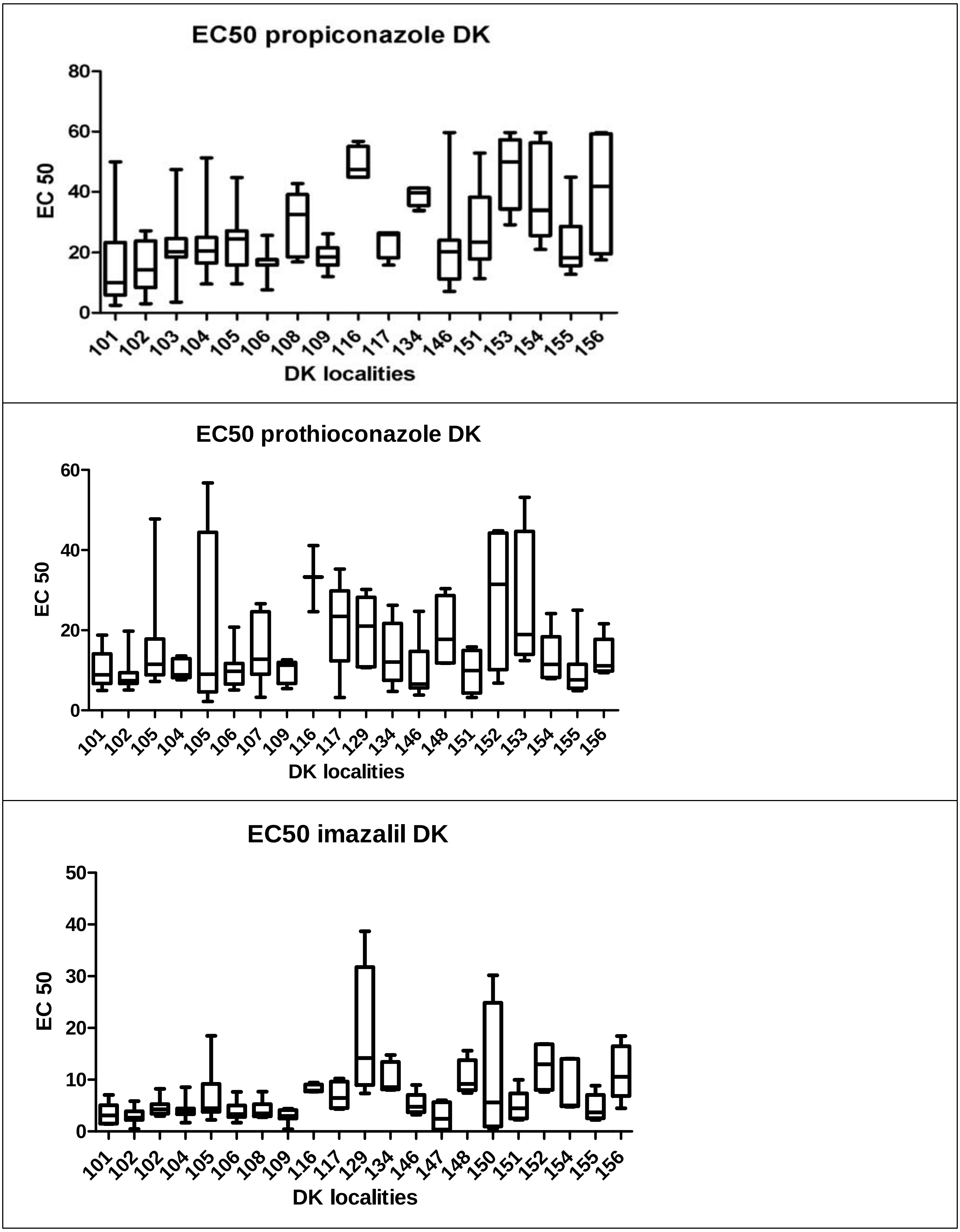


Figure 7. EC_{50} for propiconazole, prothioconazole and imazalil for Danish localities in 2008. The distribution of EC_{50} values is illustrated with box and whisker plots. No significant differences between localities were measured.

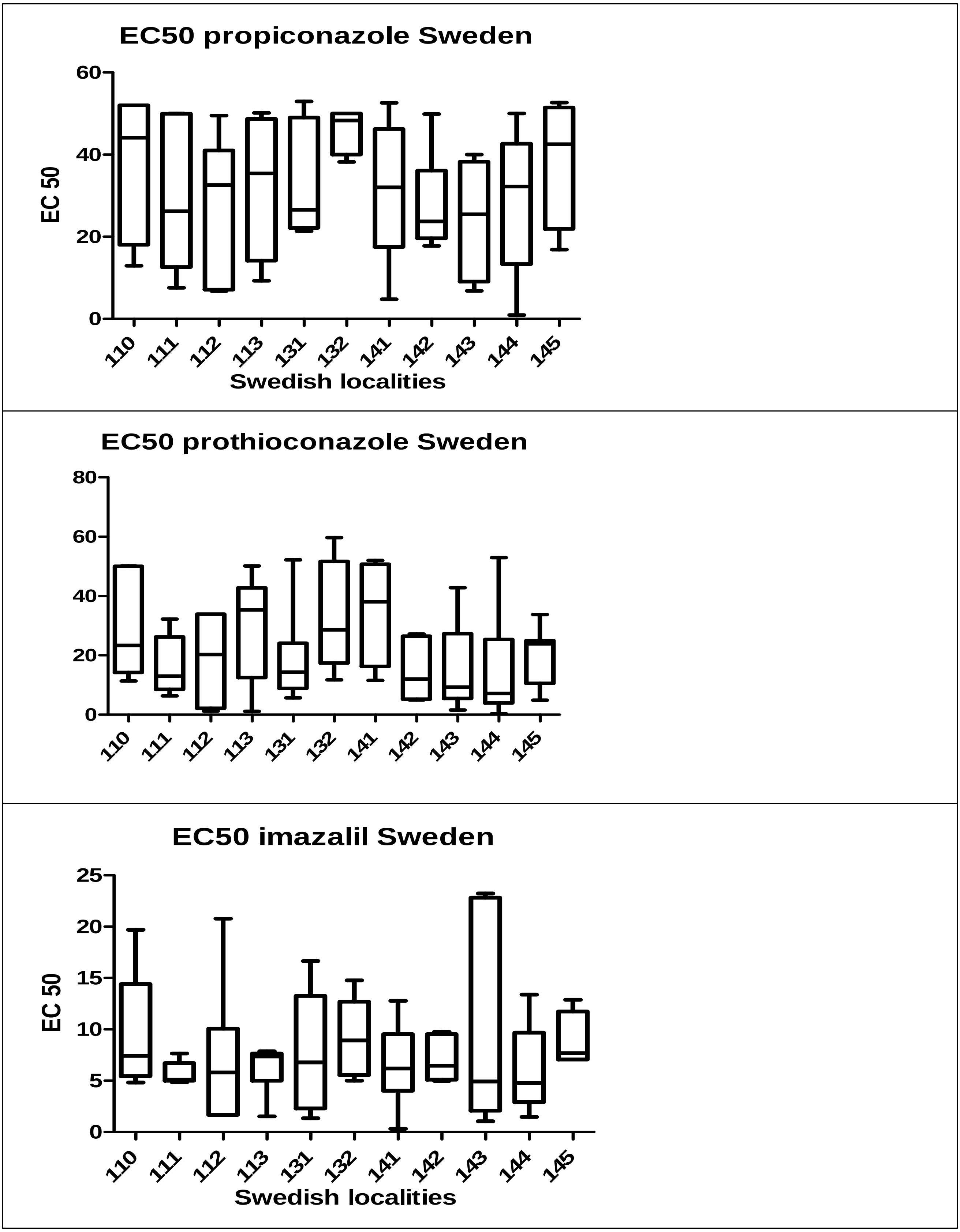


Figure 8. EC_{50} for propiconazole, prothioconazole and imazalil for Swedish localities in 2008. The distribution of EC_{50} values is illustrated with box and whisker plots. No significant differences between localities were measured

Strobilurin resistance in the Drechslera tritici-repentis population

In 2008 leaf samples from 9 localities were collected at GS 65-75 and tested for strobilurin resistance using PCR-based methods carried out by BASF. The samples were analysed for 3 mutations as indicated in Table 8. All 3 mutations are common in the Danish population although G137R is least frequent. Two field trials were sampled both spring and summer and results did not show a great degree of consistency between occurrences of mutations at these two timings. The survey for resistance in two fields at GS 75 after different fungicide treatments had taken place similarly showed major variation within the field. Only slight changes had taken place during the season following input from different fungicide groups, e.g. the strobilurins (see Table 9).

All 3 mutations were found to be present in the same field although a sample may show dominance of one or the other. Results from 6 years of monitoring in Denmark are summarised in Table 10 which shows that F129L and G143A are present in approximately 40-50%.

Table 8. Frequence of strobilurin resistant mutations in the DTR population analysed at 9 Danish localities in 2008.

Locality	Timing	F129L	G137R	G143A
Flakkebjerg	**Spring**	**27**	**7**	**20**
Hyllested	**Spring**	**100**	**6**	**0**
Mørkøv	**Spring**	**63**	**9**	**10**
Vipperød I	**Summer**	**0**	**0**	**100**
Vipperød II	**Summer**	**0**	**0**	**79**
Hjerm	**Summer**	**72**	**0**	**12**
Brønderslev	**Summer**	**38**	**32**	**X**
Hobro	**Summer**	**76**	**0**	**32**
Landbrug syd	**Summer**	**71**	**0**	**31**
Flakkebjerg	**Summer**	**16**	**1**	**56**
Hyllested	**Summer**	**32**	**5**	**66**
Average	**Summer**	**41**	**5**	**48**

Localities with dominance of G143A in the *D. tritici-repentis* population are expected to be less well controlled from strobilurins, whereas F129L and G137R are expected to have less impact on field performance.

For both strobilurins and triazoles (prothioconazole, propiconazole) major differences in sensitivity between isolates and localities have been seen over the years (table 10). No triazole testing was carried out in 2008, as the low disease pressure only gave rise to small lesions which are difficult to isolate spores from.

Table 9. Average level of strobilurin resistance in samples collected from the two localities. The figures are an average of 2 replicates.

	Flakkebjerg			Hyllested		
	% resistance (F129L)	**% resistance (G137R)**	**% resistance (G143A)**	**% resistance (F129L)**	**% resistance (G137R)**	**% resistance (G143A)**
Spring sample before application	27	7	20	100	6	0
Samples after application						
1. Platoon	22	0	37	6	0	92
2. BAS 62700F	17	0	58	45	5	72
6. BAS 54900F + Platoon	14	0	83	42	0	64
7. BAS 62700F + Platoon	13	0	48	34	0	64
8. BAS 62700F + Input	14	0	28	22	13	58
9. Juwel TT	9	0	60	10	0	82
10. Opera N	13	0	82	40	11	54
11. Opus	6	0	85	28	15	59
12. Tilt 250	19	5	44	30	16	65
13. Input	9	0	12	38	0	66
14. Fandango + Input	30	0	89	40	0	70
15. Amistar Opti + Gladio	35	0	49	33	10	63
Untreated	31	0	40	45	0	53
Average	16	0,6	57	33	5	67
Average with strobes	19	0	64	29	3	70
Average without strobes	16	1	45	34	8	62

Table 10. Summary of results from fungicide resistance testing in *D. tritici-repentis* since 2003. Data summarise results fom both triazoles and strobilurins.

	No of isolates and localities ()	EC_{50} propiconazole	EC_{50} prothioconazole	EC_{50} azoxystrobin	% mutations of G143A	% mutations of F129L
2003	2				0	2
2004	47				2	28
2005	70 (9)			11.5	48	30
2006	39 (7)	6.6	16.3	11.7	47	33
2007	50 (7)	3.1	11.1	17.6	45	33
2008	(10)				48	41

Strobilurin resistance powdery mildew in barley

Since the late 1990s strobilurin resistance in the wheat population of powdery mildew has been common. Surprisingly, only minor changes have previously been found in the powdery mildew population in spring barley, which might be linked to the fact that mildew is relatively less common today where most cultivars have Mlo resistance. In 2008 11 samples were collected from spring barley and analysed for G143A mutations by BASF. 4 of the tested samples had relatively high levels of resistance to strobilurins. One of the samples was taken after 2 treatments with strobilurins had taken place. This gave rise to 40% resistance in the population and a low field performance in the trial (see Figure 6)

Even though probably quite high proportion of the population still is sensitive the use of strobilurins for control of mildew is not regarded as reliable. Only mixtures with other mildew active substances are recommended.

Table 11: Per cent resistance in the powdery mildew population, assessed as % G143A mutations.

Sample no.	Locality	Treatment	Cultivar	% mutation G143A
1	Flakkebjerg	Untreated	Sebastian	**12**
2	Flakkebjerg	Untreated	Power	**0**
3	Flakkebjerg	Untreated	Cork, trial 08341-2	**0**
4	Flakkebjerg	2 x 0.5 l/ha Comet	Cork, trial 08341-2	**41**
5	Grindsted	Unknown	Proctor	**0**
6	Grindsted	Unknown		**0**
7	Boelshoj	Unknown		**10**
8	Karise	Unknown		**0**
9	Tystofte	Unknown		**17**
10	Askov	Unknown		**0**
11	Askov	Unknown		**0**

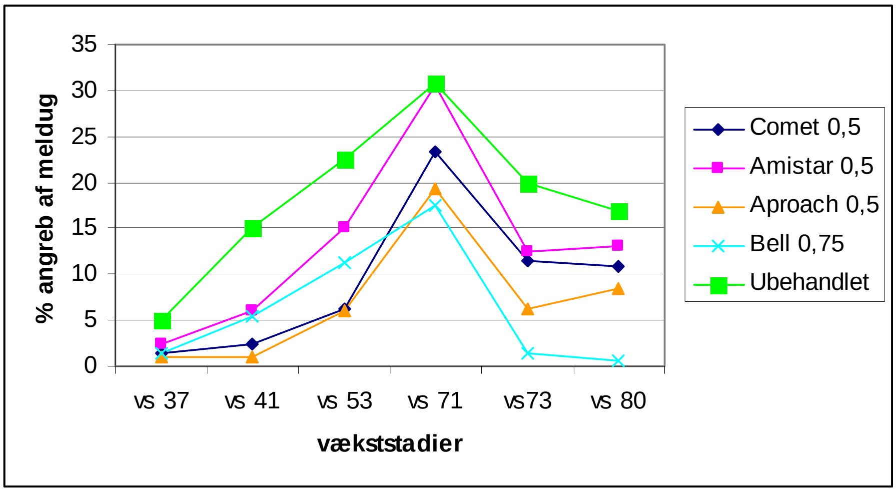


Figure 6. Attack of powdery mildew in the spring barley cultivar Cork (08341-2). The trial was sprayed twice (GS 31 & 39) with half rate of strobilurins and Bell. Initially, the best effect was obtained from Comet and Aproach. After GS 71 the relatively weak mildew product Bell was found to give best effect, indicating a shift. Mildew sampled from untreated plots showed no sign of resistance whereas mildew following 2 x 0.5 l Comet had 41% strobilurin resistance.