Project title	Are (in)sensitivities of scab isolates to different fungicides correlated?
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AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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31 March 2011

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GROWER SUMMARY

Headline

- Mixed (or alternate) use of captan, dodine (Radspor) and pyrimethanil (Scala) with other scab fungicides can be generally recommended to maintain scab control and minimise the risk of resistance development.

Background and expected deliverables

One consequence of overusing fungicides is the selection of fungal strains less sensitive (a more accurate term than more resistant) to the fungicide actives ingredients used. Disease management strategies have been developed to reduce the risk of emergence and spread of these insensitive fungal strains. Overusing DMI fungicides has led to emergence of scab strains less sensitive to DMI fungicides in USA and Canada. In the long-term, this may lead to loss of disease control. There is some anecdotal evidence that isolates from DMI-sprayed orchards in the UK appear to have an overall reduction in sensitivity to myclobutanil (Systhane), which is commonly observed in other regions. However, failure to control scab control is often due to poor spray timing and not reduced sensitivity.

Some recent studies have shown that cross-resistance of the scab fungus to fungicides may or may not exist depending on the particular fungal populations concerned. If reduced sensitivity to one fungicide exists, then care is needed to select alternative product(s) without jeopardising disease control and resistance management. However, the necessary knowledge on cross-resistance required to make such rational selection decisions is not available yet.

Recent Canadian research suggests independent resistance mechanisms to myclobutanil and kresoxim-methyl (Stroby) but a positive correlation in resistance to myclobutanil and flusilazole. This result does not agree with results obtained in New York State. More recent research on other pathogens also suggests that the presence and the extent of cross-resistance depends on particular fungal populations and fungicides concerned. It is, therefore, necessary to carry out research for each particular fungal population to understand the potential of cross-resistance to particular fungicides. In any anti-resistance strategy, information on cross-resistance is critical for devising control strategies in cases where reduced sensitivities to one fungicide have been observed. There has been no published information for the baseline sensitivity and the current status of sensitivity to common scab fungicides in the UK scab population.

Expected deliverables and benefits

This project will generate information on the baseline sensitivity of the UK apple scab population to important scab fungicides registered in the UK. This information is essential for future research and development on scab insensitivity to fungicides.

The project will generate information on scab (in)sensitivities to common scab fungicides, which can be used to re-assess the extent of the shift towards insensitivities against each fungicide. The information on the rate of insensitivity shift is critically important to reappraise the long-term future of each fungicide; this is particularly important because of potential reductions in the number of fungicides in the EU. Information on the presence or absence of cross-resistance among these fungicides could potentially allow growers to develop more sustainable anti-resistance strategies to control scab. For example, if possible, fungicides that exhibit no or low cross-resistance should be used on a given site. This project will generate the range of concentration of each fungicide deposited on leaf surfaces following an application. This information is essential for interpreting *in vitro* sensitivity results in terms of field control efficacy.

Summary of the project and main conclusions

The responses of many isolates to nine active ingredients of eight scab fungicides registered in the UK have been quantified and the level of fungicide re*sidues o*n leaves has also been estimated following a standard application of these fungicides.

In this study scab isolates with reduced sensitivity to captan did not show a related reduced sensitivity to other scab fungicides i.e. there was no correlation. Similarly, scab sensitivity to boscalid (a component of Bellis) and dodine (Radspor) were not statistically significantly correlated with reduced sensitivity to the other scab fungicides trialled.

However, the sensitivities of scab isolates to some of the other fungicides in the trial were statistically significantly correlated, meaning that scab isolates that showed reduced sensitivity to one also showed reduced sensitivity to another and vice versa as shown in Table 1.

Table 1. Statistically significant correlation (at the level of 5%) between the reduced sensitivity of scab to different fungicide actives

	Cyprodinil	**Dithianon**	**Fenbuconazole**	**Myclobutanil**	**Pyraclostrobin**	**Pyrimethanil**
Cyprodinil (Switch)	X	X		X	X	X
Dithianon (component of Maccani)	X	X	X	X	X	
Fenbuconazole (Indar)		X	X	X		
Myclobutanil (Systhane)	X	X	X	X	X	
Pyraclostrobin (component of Bellis and Maccani)	X	X		X	X	
Pyrimethanil (Scala)	X					X

X means that a significant correlation in scab sensitivity was found between the fungicide in the column and the fungicide in the row and so they should not be used sequentially in the spray programme.

The findings shown in Table 1 mean that care should be taken when using fungicides in the programme where significant correlation in scab sensitivity has been found. To minimise the risk the programme should include captan, dodine (Radspor) and pyrimethanil (Scala). Where the other fungicides are used they should be alternated with either captan, dodine (Radspor) or pyrimethanil (Scala) as appropriate.

Initial fungicide deposition concentration on leaves, following application at the full recommended rate at 200 L/ha, was higher than the concentration at which reduced sensitivity was reported. Heavy rainfall resulted in a significant loss of all fungicides, except dithianon on leaf surfaces.

For best effect the spray interval, and possibly the rate, needs to be adjusted according to the past/predicted rainfall and the rate of host growth.

Financial benefits

Scab can cause serious losses, particularly in some seasons when weather conditions favour it and limit opportunities to spray. Damaged fruit is unmarketable and so effective control of the disease is vital. The findings of this project will encourage growers to use the most suitable spray programmes that give effective control and minimise the establishment and subsequent spread of scab strains that are insensitive to fungicides.

Action points for growers

- Growers should think carefully the selection of fungicides in the spray programme to avoid frequent and repeated use of those fungicides to which reduced sensitivities of scab is known to be highly positively correlated.

- Growers need to adjust the spray interval (and possibly the rate) according to the past/predicted rainfall and the rate of leaf and fruit growth.

SCIENCE SECTION

Introduction

Apple scab (*Venturia inaequalis* (Cke.) Wint.) is one of the most economically important diseases of apples worldwide. The disease attacks leaves, flowers, fruits, shoots and bud scales. Scab control is usually achieved by the use of fungicide sprays applied routinely from bud burst at 7-10 day intervals until the risk of scab ceases. Demethylation inhibitors (DMI) fungicides are probably the most important group of fungicides for scab control in the UK because of their curative action against scab and their control of powdery mildew*,* which in the UK is the next most important disease of apples. Overuse of DMI's in some countries has led to selection and subsequent establishment of *V. inaequalis* strains that are less sensitive to DMI's (Stanis & Jones, 1985; Thind *et al.*, 1986; Hildbrand *et al.*, 1989; Braun & McRae, 1992; Köller *et al.*, 1997; Jobin & Carisse, 2007). Resistance to DMIs is believed to be quantitative (Georgopoulos & Skylakakis, 1986; Köller & Scheinpflug, 1987; Smith *et al.*, 1991); consequently, the loss of sensitivity by the pathogen tends to be gradual.

Information on cross-resistance is critical for devising control strategies where reduced sensitivities to one fungicide are observed. For *V. inaequalis*, there appears to be a lack of cross-resistance among dodine, the benzimidazoles and the DMIs. The efficacy of scab control achieved with benzimidazoles in orchards with fungal strains resistant to dodine was high (Jones, 1981), and DMIs controlled scab effectively in orchards located within a region known for widespread dodine and benzimidazole resistance (Wilcox *et al.*, 1992). Recent research has indicated, however, that the mechanisms of resistance to DMIs and dodine fungicides might not be entirely independent (Köller & Wilcox, 1999, 2000). Isolates of *V. inaequalis* selected for phenotypic traits of resistance to dodine or benomyl remained as sensitive to other classes of fungicides; but isolates that were already resistant to dodine were prone to accelerated adaptations to other fungicides.

In the UK most apple growers apply spray programmes made up of combinations of fungicides which differ in mode of action in order to achieve better control and minimise the risk of fungicide resistance. The use of DMI fungicides in the programme has increased in recent years, mainly because of the lack of alternative products to control powdery mildew. Two common DMI fungicides used in the UK to control scab are Systhane (a.i. myclobutanil) and Indar (a.i. fenbuconazole). The former is more widely used because of its effects against powdery mildew. There is some evidence for reduced sensitivity to myclobutanil in the UK (Roberts & Crute, 1994; Gao *et al.*, 2009a). Nevertheless, poor scab control in sprayed orchards can usually be attributed to poor spray timing and/or extended spray intervals due to unfavourable weather (Berrie, pers comm.). Due to the lack of alternatives in controlling scab and powdery mildew, growers sometimes continue to use DMI fungicides, even in orchards with reduced sensitivity to DMIs. There has been no published study yet on the cross-resistance between important fungicides that have been registered for scab control in the UK as well as baseline sensitivities to these fungicides.

Materials and methods

Collection of samples

Samples of leaves or fruit with actively sporulating lesions of apple scab were obtained from various orchards in the summer of 2009 via several consultants. These orchards had all experienced problems with scab control. In addition, samples from a 50 year old mixed orchard were considered to exhibit the baseline population since this orchard with three cultivars (Cox, Worcester and Bramley) has never received any fungicides. Moreover, we have also included several isolates that are known to have differing degrees of insensitivity to myclobutanil from previous studies (Gao *et al.*, 2009a). Note that this project is not about the actual insensitivity of scab to different fungicides but about the correlation in the insensitivity to them. If the focus was on the emergence of fungicide sensitivity a different sampling scheme would have been be used to sample isolates from orchards with a range of spray programmes. Reliable baseline data against which it is possible to assess the level of insensitivity is only available for myclobutanil and, to a lesser extent, fenbuconazole of the fungicides tested.

Once delivered to the lab at EMR, actively sporulating lesions were cut out from the leaves or fruit using a cork borer. The lesions were then laid out on paper towels and allowed to air dry for 48 h. The scab lesions were individually placed into a micro-centrifuge tube, and stored at -18°C until required.

Obtaining single-spore isolates

In order to produce more reliable test results, we have isolated single spore isolates from sampled leaf discs. We aimed to obtain 10-15 single spore isolates for sampling site. Water agar (WA, 15 g/L), amended with rifamycin (50 mg/L) (Sigma-Aldrich. Poole, UK) after autoclaving, was used to obtain single-spore isolates. An infected leaf disc (stored at -18°C) was added to deionised water and agitated thoroughly to release conidia. Conidial suspensions were adjusted to $8x10^3$ per ml and 200 µl of suspension pipetted onto the rifamycin-amended WA plates and spread evenly. After incubation at 15°C for 24 h, individual germinated spores were excised using a needle under a microscope in a laminar flow cabinet and placed on potato dextrose agar (PDA) plates. Plates were incubated at 15°C and, when large enough, each colony was transferred to a fresh PDA plate.

In vitro testing for fungicide sensitivity

Fungal isolates were tested for sensitivity to all registered scab fungicides in the UK (Table 1). For those fungicides with two active substances, we have obtained formulated products with each active for ease of data interpretation. Thus, fungicides Kayak, Filan and Vivid were used for testing cyprodinil, bosacalid and pyraclostrobin. The concentrations for testing were determined from published studies on scab or other pathogens and varied with fungicides (Table 1). For each fungicide, five or six concentrations were used. A stock solution for each fungicide was prepared and an appropriate amount of the stock solution was used to amend water agar to produce agar plates with required concentrations of each fungicide. Rifamycin was also added to the agar (0.05 µg/ml) to reduce bacterial contamination. Two types of tests were carried out in order to understand the protectant and curative efficacy.

Protectant effect*:* We carried out this test for boscalid and captan. A suspension of *V. inaequalis* conidia was made by washing fungal plates with 2 ml deionised water and adjusted to approximately 5000 conidia per ml using a haemocytometer where possible. A 10 µl drop of each spore suspension was placed in the centre of each agar plate and evenly spread over the agar surface of the centre region using a sterile rod. The plates were incubated at 20°C for 24 h before the percentage of germination was recorded under a microscope (Leitz, model Laborlux S). Because of lack of sporulation in many single-spore isolates (which is a common phenomenon for apple scab), we have also used spores washed from leaf discs directly for this test.

Table 1. List of fungicides and their active(s) and testing concentrations (ppm, mg/L) used for testing scab isolates

Product	**a.s.**	**Mode of action**	**FRAC code**	**Concentration**					
				C1	**C2**	**C3**	**C4**	**C5**	**C6**
Systhane	myclobutanil	Curative (DMI)	G1(3)	0	0.01	0.1	0.5	2	5
Indar	fenbuconazole	Curative (DMI)	G1(3)	0	0.01	0.05	0.25	0.5	1
Switch	cyprodinil	Both (AP)	D1(9)	0	0.001	0.01	0.1	0.5	1
	fludioxonil	Protectant (PP)							
Captan	captan	Protectant (MSC)	M4	0	0.05	0.25	1	5	20
Maccani	dithianon	Both (?) (MSC)	M9	0	0.01	0.1	0.5	2	5
	pyraclostrobin	Both (QoI)	11	0	0.001	0.01	0.1	1	5
Bellis	bosacalid	Protectant (SDHI)	7	0	0.001	0.01	0.1	1	5
	pyraclostrobin								
Scala	pyrimethanil	Both (AP)	D1(9)	0	0.01	0.5	0.25	1	4
Radspor	dodine	Both (MSC)	U12	0	0.05	0.2	0.5	1	4

Curative effect: This test was carried out for all fungicides except boscalid and captan. Mycelial plugs of 4 mm size were cut from each isolate and placed on to the agar plates with each concentration of specific fungicide (Table 1). For each combination of concentration, isolate and fungicide, there were two replicate plates and on each plate there were three mycelial plugs that were 5 cm from each other. The plates were incubated at 20°C in the dark. The length and width of each colony were both measured after 4-5 weeks incubation.

In addition to the mycelial plug test, we have also carried out a test based on germ tube elongation. A suspension of *V. inaequalis* conidia of a test sample (leaf disc) was prepared and adjusted to approximately 5000 conidia per ml using a haemocytometer. A 10 µl drop of each spore suspension was then placed in the centre of an agar plate and evenly spread over the agar surface using a sterile rod. The plates were then incubated at 20°C in darkness for 48-64 hours. After incubation, the growth of fungal hyphae was assessed. Where a conidium developed multiple germ tubes, only the longest hypha was recorded. Assessment of a single sample at all concentrations was completed within 60 min. Hyphal length was measured using a graticular eyepiece placed in the eyepiece of a microscope (eyepiece magnification X10, objective magnification X10); 200 intersections on the graticular eyepiece are equal to 10 mm. A germinated spore was then randomly selected from the view field and hyphal length was estimated.

Data analysis: In several cases where the fungal growth/development was much greater in fungicide-amended plates than in untreated plates, we substituted the fungal development without fungicides with the larger development value. ED_{50} values were estimated by fitting an exponential or a linear by linear quotient model to the fungal growth data (adjusted for initial plug size). It is not possible to fit a single model type to all the data sets; thus, the better of the two models (on the basis of the percentage of variance accounted for) was chosen for each sample to estimate the ED_{50} value. For a few data sets, both models failed to fit the data but the fungal development was clearly reduced by more than 50%; we used the simple linear interpolation to estimate the ED_{50} value. In several other cases, it was not possible to estimate the ED_{50} value and fungal growth was only reduced slightly within the fungicide concentration tested. For these cases we simply indicate that the ED_{50} value was greater the larger the concentration tested. Both normal and Spearman correlation coefficients were calculated between ED_{50} values for each pair of fungicides. Genstat (Payne, 2006) was used in all analyses.

Estimating concentration of fungicide deposition in field conditions

In order to interpret the *in vitro* findings in terms of field control efficacy, we need to know the approximate range of concentrations of fungicide deposited on the leaf surface. A tank mix of these fungicides was prepared at the full recommended rates and applied to a block of apple trees at the water volume of 200 L/ha. Due to compatibility issues between the fungicides, the fungicides were applied on three separate occasions within a short interval on 23 September 2009 and 30 September 2010 on cv. Gala (TL161) at East Malling Research. The first application was Radspor (1.5 L/ha), the second a tank mix of Dithianon (1.1 L/ha), Bellis (0.8 kg/ha) and Scala (1.125 L/ha), and the third the mix of Systhane (0.45 L/ha), Switch (0.8 kg/ha), Indar (1.0 L/ha) and Captan (2.9 kg/ha). Fungicides were applied until the previous spray had dried.

On the same day after spray deposits had dried, 10 samples (each with two leaves) were randomly sampled from different tree canopy zones: outside, top and inside. Additional 10 samples of leaves were obtained on 1 October. The total area of each sample (two leaves) was measured and samples were then sent to the QTS ltd. for quantification of individual active substances.

Results

Isolate collection

We have obtained a number of samples of leaves and fruit with scab lesions from several sites. From these samples we have obtained a sufficient number of single-spore isolates for testing. A summary of the isolates obtained is given in Table 2. In addition to these isolates from commercial orchards, we have also included 10 isolates from the baseline population (an old orchard that has never received any fungicide spray treatments) and six isolates with known degree of reduced sensitivities to myclobutanil.

In vitro sensitivity testing

General results

Collection of single spore isolates was completed by late September 2009 and we started *in vitro* testing for the protectant effects. Unexpectedly, we encountered severe contamination problems because we cannot sterilise fungicides before we add them to the agar media. It took us at least a month to find a way to reduce (though not eliminate) the contamination problem. Thus, we had to use the germ tube length test method for assessing curative effects, which was much more labour intensive and limits the number of isolates that we could test. On average, it took two days to test one single isolate. In total we have tested about 50 isolates but for only 31 isolates did we had enough non-contaminated plates at several concentrations to accurately estimate ED_{50} values for at least two fungicides (Table 3).

Table 2. Summary of single-spore scab isolates obtained in 2009

Site	Sample	Number of isolates
West Midlands A	Leaves	6
Herefordshire	Fruit	24
Kent A	Fruit	18
	Leaves	18
Kent C	Fruit	9
Kent B	Leaves	16
	Fruit	36
West Midlands B	Fruit	9
	Leaves	9
East Malling (TL161)	Leaves	4
	Fruit	9
Total		158

Of the 31 isolates, three isolates (names starting with 05 [see Table 3]) were from an old mixed orchard (ca. 50 years old) that had never received any fungicide spray, four were from a commercial orchard with no scab control problem, and all the remaining 24 isolates were from orchards where scab control was problematic (9 isolates sampled in 2007 from an orchard in the West Midlands – name starting with 07 [see Table 3], and the other 15 isolates sampled in 2009 – Table 1).

Of the 9 active substances tested, boscalid had the fewest number of isolates (8) and myclobutanil had the greatest number of isolates (29). Of the total 170 estimated ED_{50} values, 15 cannot be accurately determined from the current data (Table 3), six of which were for myclobutanil. Of all the isolates tested, there was only a single isolate that had high ED_{50} values against at least four products: myclobutanil, fenbuconazole, dodine and dithianon. It was not rare to have isolates with high ED_{50} values against two products.

Correlation in ED_{50} values

Table 3 presents the estimated ED_{50} values against all nine products. As pointed out previously, the project focus is on the correlation among the estimated ED_{50} values instead of their actual magnitudes. For at least half of the samples from problematic orchards in 2009 the level of insensitivity to myclobutanil has increased 10 times compared to previously reported baseline values.

Correlation among ED_{50} values is given in Table 4. Of the 26 pairwise correlations, only nine were statistically significant. The nine products can be divided into two groups: (1) boscalid, captan and dodine, and (2) cyprodinil, dithianon, febuconazole, myclobutanil, pyraclostrobin and pyrimethanil. ED_{50} values for the first group are not significantly correlated with those for any active substance whereas ED_{50} values of any given substance in the second group is significantly correlated with at least one substance within the group (Table 2). Within the second group, the undetermined ED_{50} values for any given substance appeared to be associated with higher ED_{50} values for the other substances in the group (Table 4). Thus, the correlation in ED_{50} values among these six substances might be even higher if ED_{50} values of these isolates had been determined.

Figure 1 plots the pairwise ED_{50} values for the active substances in the second group. Of the six substances, ED_{50} values for pyrimethanil was significantly correlated with cyprodinil only ($r = 0.809$). For cyprodinil, myclobutanil and dithianon, their ED_{50} values were significantly correlated with four other substances. For pyrimenthanil, fenbuconazole and pyraclostrobin, their ED_{50} values were significantly correlated with one, two and three other substances, respectively.

Estimating concentration of fungicide deposition in field conditions

Table 5 presents the summary of fungicide deposition immediately after spray and eight days after spray in 2009. As expected, there is large variation in the spray deposition. For example, the concentration for captan on 23/09/09 ranged from 4.98 to 27.00 µg/cm^2.

There are some reductions in the concentration from day 0 to day 8 but varied greatly with fungicides (there was no rainfall between 23/09 and 01/10). For dodine, there were no reductions at all; in contrast for myclobutanil the reduction was nearly 47%. Of the nine active substances tested, four showed significant ($P < 0.05$) reductions from day 0 to day 8: cyprodinil, myclobutanil, pyraclostrobin and pyrimethanil.

If we assume that 0.04 ml is needed to fully cover 1 cm^2 leaf surface (based on lab trials), we can estimate the concentration of each active ingredient on the leaf surface as ppm, which can then be used to interpret *in vitro* test results. Alternatively, we could also assume a varying degree of leaf coverage at the time of spray, we could then estimate the water deposition (200 L/ha) on 1 cm^2 leaf surface to be in the range of 0.01 to 0.05 ml. Figure 2 shows the range of fungicide concentrations on the leaf surface assuming 0.04 ml of water to wet 1 cm^2 leaf surface.

Table 3. Estimated ED_{50} values (ppm) of tested products at which fungal germination or germ tube is reduced by 50%

Isolate	Boscalid	Captan	Cyprodinil	Dithianon	Dodine	Fenbuconazole	Myclobutanil	Pyraclostrobin	Pyrimethanil
05_320	0.004	1.0	0.0001	0.008	0.1	0.014	0.003	0.001	0.1164
05_340	0.053	0.114	0.01	0.003	1	0.022	0.021	0.001	0.01
05_354	0.1	0.065	0.005	0.689	0.954	0.825	> 2	0.01	0.002
06_003	1.669	0.096		0.05			0.0004	0.001	
06_034	0.010	> 20	0.004	> 2	0.168		0.028		
06_053	0.002	0.08	1.229	1.719	0.237	0.25	0.041	0.0004	
06_112			0.012	0.522	0.327	0.003	0.01	0.081	0.379
07/064Short1					1.803	0.822	2.955	0.029	1.076
07_064inter5			0.739	3.012	4.471	0.143	2.623	0.075	0.907
07_064Long5				4	2.557	1.007	> 5	2.1	0.504
07_064short8			1.082	> 5	> 4	> 1	> 5	1.921	1.055
07_065Inter6			0.685	7.656	0.099	> 1	7.494	1.022	0.615
07_065long_4			0.446	3.887	0.14	0.909	5.079	0.609	0.738
07_065long10			1.084	8.082	0.224	0.661	4.93	0.521	0.774
07_065long4			0.212	0.406			0.01	0.002	0.361
07_065short1			0.001	0.004	1.329	0.006	0.006	0.001	0.287
09_001	0.509	8.054	0.0003	0.286	0.123		0.707		
09_003				3.4	0.206	1.0	0.015	0.001	0.335
09_006				1.149	0.185	0.90	0.166	0.126	0.165
09_031(6)				3.894	0.156	2.02	4.722	> 4	> 5
09_032(4)					0.805				
09_034(2)			> 1	5.168	0.321	0.987	4.841		3.721
09_035(2)					1.359				
09_036(4)			1.21		0.432	0.221	2.652	1.16	2.005
09_037_5		0.437					> 2		
09_31_4	0.202	1.609	0.1	0.018	0.056	0.105	0.1	0.035	0.9065
09_38_1		0.268					0.021		
09_38_2		0.731					> 2		
09_38_3		0.276					1.956		
09_38_4		0.343					1.056		
09_38_5		1.476					> 2		
Total samples	**8**	**14**	**17**	**21**	**23**	**19**	**29**	**20**	**19**

Table 4. Correlation among ED_{50} values of apple scab against nine active substances in potential scab fungicides; * and ** indicate significance at the level of 5% and 1%, respectively.

a.s.	Boscalid	Captan	Cyprodinil	Dithianon	Dodine	Fenbuconazole	Myclobutanil	Pyraclostrobin
Captan	**0.057**							
Cyprodinil	-0.288	-0.284						
Dithianon	-0.305	-0.165	0.719**					
Dodine	-0.234	-0.453	0.094	-0.014				
Fenbuconazole	0.158	-0.461	0.236	0.556*	-0.144			
Myclobutanil	0.071	0.116	0.524*	0.892**	0.005	0.611*		
Pyraclostrobin	-0.146	0.763	0.625*	0.589*	0.142	0.362	0.774**	
Pyrimethanil	0.829	0.88	0.809**	0.417	-0.046	0.23	0.471	0.438

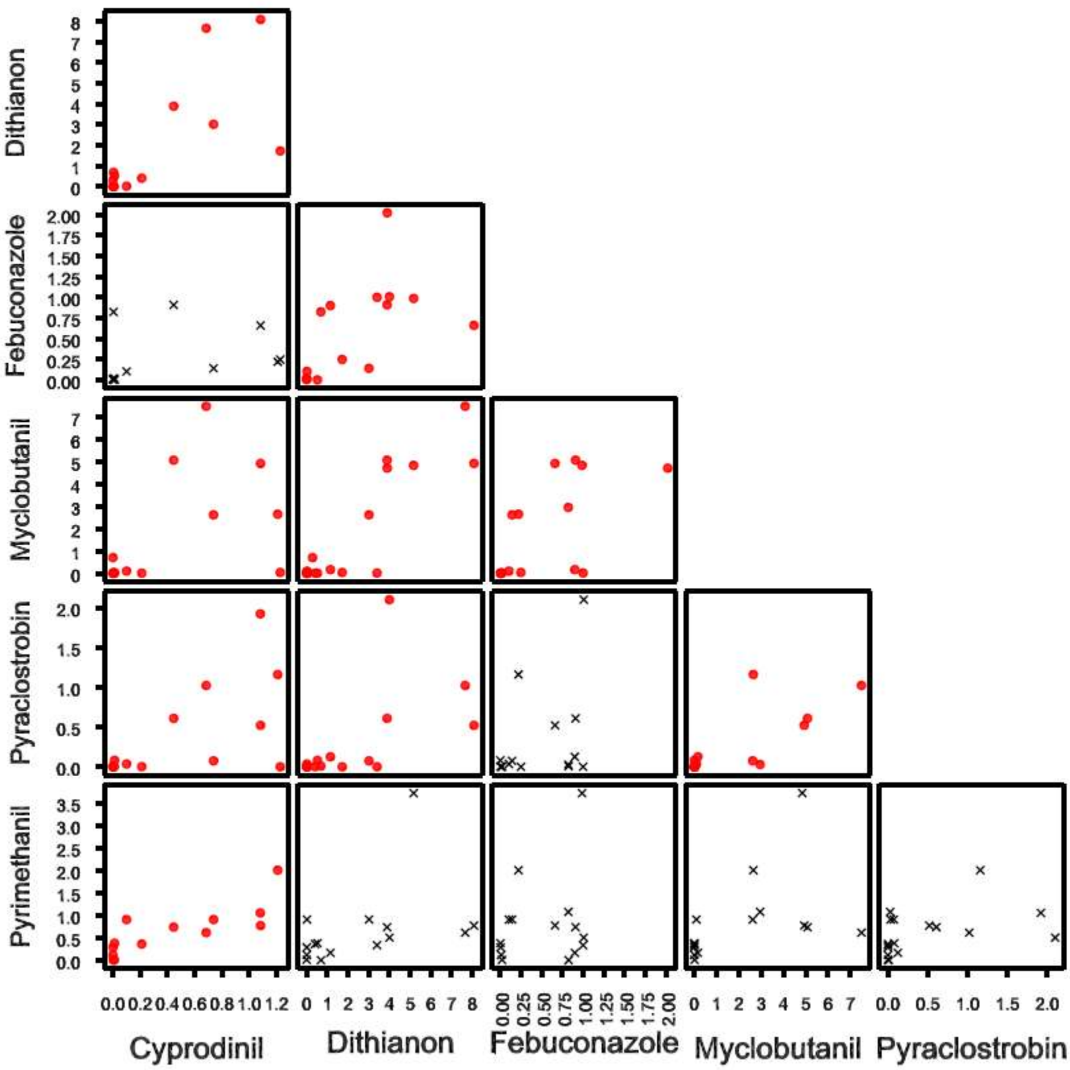

Figure 1. Plot of pairwise Estimated ED_{50} values (ppm) among the six active substances: Cyprodinil, Dithianon, Fenbuconazole, Myclobutanil, Pyraclostrobin, Pyrimethanil. The plot with filled circles indicates that the correlation is statistically significant at the level of 5%

Table 5. Estimated fungicide concentration (μg/cm^2 leaf area) immediately (23/09/09) and eight days (01/10/09) after spray; * indicates the significance at the level of 5%

Date	Boscalid	Captan	Cyprodinil	Dithianon	Dodine	Fenbuconazole	Myclobutanil	Pyraclostrobin	Pyrimethanil
23-Sep	0.66	8.71	0.27	0.44	0.31	0.15	0.49	0.31	0.20
23-Sep	0.82	15.07	0.57	0.56	0.48	0.34	1.04	0.38	0.25
23-Sep	0.84	16.73	0.41	0.62	0.44	0.25	0.74	0.38	0.29
23-Sep	0.29	4.98	0.23	0.23	0.13	0.09	0.33	0.11	0.10
23-Sep	0.34	5.93	0.17	0.22	0.14	0.16	0.45	0.14	0.06
23-Sep	0.73	15.72	0.61	0.52	0.69	0.31	0.98	0.33	0.22
23-Sep	0.41	10.99	0.52	0.32	0.49	0.19	0.67	0.18	0.21
23-Sep	0.79	8.45	0.29	0.53	0.36	0.22	0.47	0.50	0.26
23-Sep	1.03	11.37	0.42	0.66	0.62	0.31	0.88	0.53	0.22
23-Sep	1.33	27.00	0.81	0.70	1.29	0.46	1.25	0.63	0.36
01-Oct	0.35	5.20	0.17	0.21	0.41	0.12	0.34	0.15	0.21
01-Oct	0.52	7.36	0.23	0.44	0.67	0.13	0.40	0.22	0.13
01-Oct	1.30	21.26	0.51	0.72	0.36	0.31	0.68	0.45	0.27
01-Oct	0.41	6.07	0.10	0.39	0.39	0.09	0.21	0.13	0.05
01-Oct	0.65	17.06	0.36	0.49	0.66	0.26	0.57	0.20	0.09
01-Oct	0.31	3.29	0.09	0.35	0.45	0.05	0.10	0.10	0.07
01-Oct	0.83	12.50	0.30	0.67	0.54	0.27	0.40	0.30	0.17
01-Oct	0.16	11.53	0.19	0.11	0.15	0.14	0.34	0.04	0.02
01-Oct	0.87	11.18	0.24	0.65	0.98	0.20	0.42	0.29	0.13
01-Oct	0.77	12.90	0.35	0.48	0.64	0.23	0.42	0.22	0.15
% reduction	14.82	13.28	41.00*	6.11	-6.09	26.74	46.91*	39.92*	40.00*

Table 6. Estimated fungicide concentration (μg/cm^2 leaf area) immediately (30/09/10) and eight days (08/10/09) after spray; * and ** indicate significance at the level of 5% and 1%, respectively

Date	Boscalid	Captan	Cyprodinil	Dithianon	Dodine	Fenbuconazole	Myclobutanil	Pyraclostrobin	Pyrimethanil
30-Sep	0.44	4.71	0.17	0.64	0.61	0.10	0.33	0.31	0.22
30-Sep	0.55	4.98	0.27	0.41	0.18	0.13	0.51	0.37	0.24
30-Sep	0.21	5.14	0.21	0.00	0.01	0.10	0.29	0.14	0.08
30-Sep	0.72	6.20	0.24	0.36	0.74	0.07	0.40	0.46	0.42
30-Sep	0.25	8.15	0.33	0.01	0.24	0.08	0.28	0.16	0.05
30-Sep	0.34	5.86	0.24	0.36	0.28	0.10	0.39	0.24	0.14
30-Sep	0.63	13.19	0.52	0.68	0.43	0.15	0.70	0.40	0.24
30-Sep	1.19	12.30	0.55	2.81	0.75	0.14	0.92	0.76	0.75
30-Sep	0.67	9.61	0.38	0.32	0.09	0.09	0.43	0.38	0.33
30-Sep	0.63	9.15	0.37	0.39	0.35	0.15	0.61	0.37	0.23
08-Oct	0.36	1.41	0.14	0.73	0.00	0.04	0.15	0.14	0.07
08-Oct	0.37	2.09	0.22	0.00	0.00	0.05	0.15	0.16	0.07
08-Oct	0.32	1.55	0.18	1.59	0.04	0.05	0.16	0.14	0.05
08-Oct	0.33	1.68	0.09	2.00	0.30	0.06	0.26	0.15	0.01
08-Oct	0.13	0.43	0.13	1.12	0.02	0.02	0.06	0.05	0.03
08-Oct	0.14	1.18	0.12	0.25	0.69	0.06	0.13	0.07	0.03
08-Oct	0.21	0.22	0.02	0.33	0.10	0.00	0.03	0.09	0.04
08-Oct	0.12	0.43	0.02	0.12	0.31	0.01	0.06	0.00	0.01
08-Oct	0.03	0.06	0.06	0.00	0.00	0.00	0.04	0.02	0.03
08-Oct	0.35	0.33	0.02	1.24	0.02	0.00	0.07	0.14	0.09
% reduction	58.03**	88.19**	69.52**	-23.16	59.63*	74.42**	77.21**	72.98**	84.26**

Residue data for 2010 are given in Table 6. The overall pattern in the residues immediately after spray was similar to 2009. There was a heavy rainfall the day after the spray. There are significant reductions in the concentration from day 0 to day 8 except for dithianon (Table 6 and Figure 3), ranging from 58% (boscalid) to 84% (pyrimethanil). However for dithianon, there were no reductions at all. Not surprisingly, correlation in the rate of reductions between two years (r = 0.48) is not statistically significant (P = 0.24).

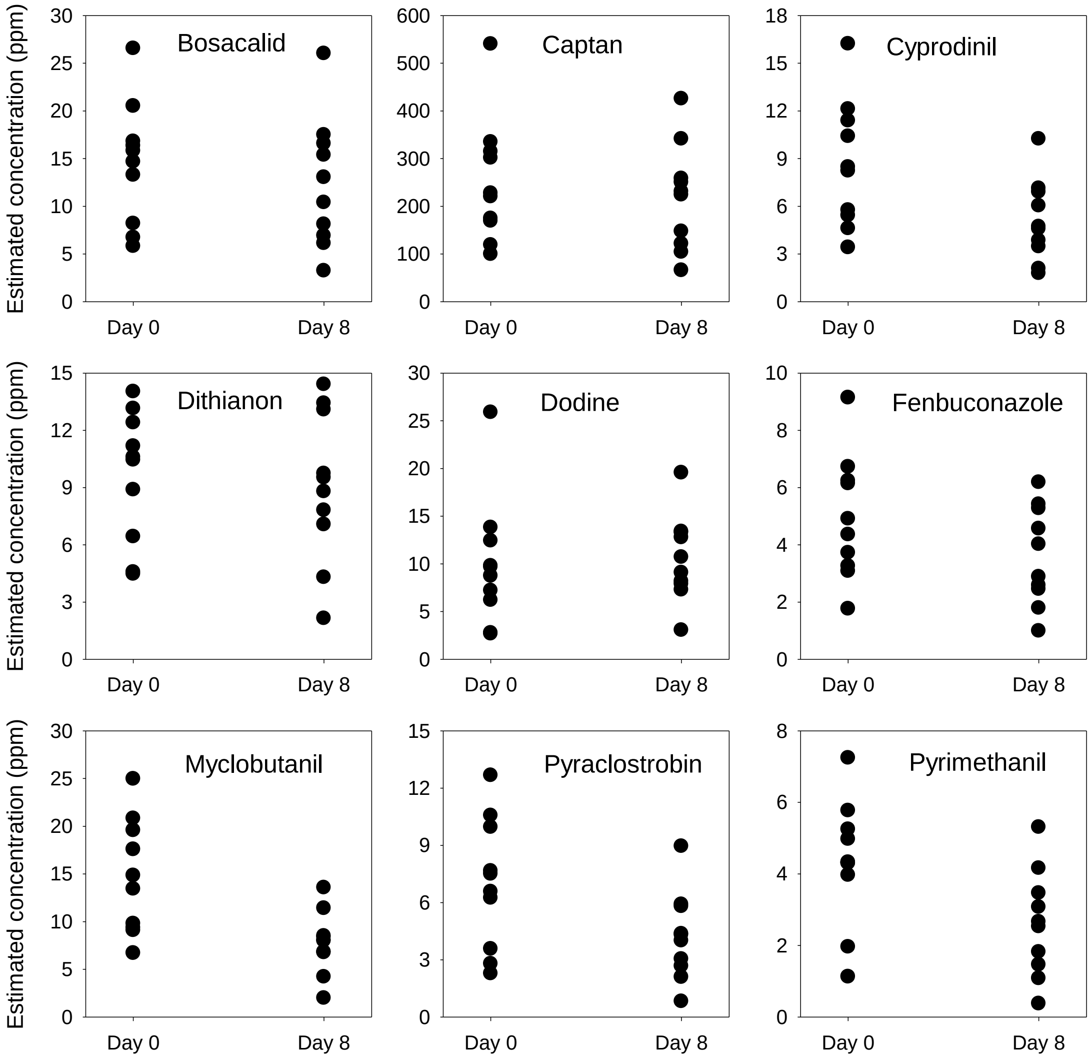

Figure 2. Estimated concentrations of active substances on the day of and eight days after the application in 2009 assuming 0.04 ml water wet 1 cm^2 of leaf surface

Discussion

General considerations

The main objective of this project is to determine the correlation among the fungal insensitivities to different scab fungicides. Thus for this purpose, we have sampled scab isolates from two extreme types of orchards: orchards without receiving scab sprays or without historical scab control problems, and orchards with known scab control problems (hence their isolates are suspected of having increased insensitivities to myclobutanil). Furthermore, because of the contamination problem which is often associated with using commercial fungicide products (since we cannot sterilise the product itself unlike when using the a.i. only), we do not have enough isolates in either of the two extreme types of orchards. Thus, the actual insensitivity levels reported in this study cannot be used to represent either the true level of fungal insensitivities or the rate of fungicide resistance development for apple scab.

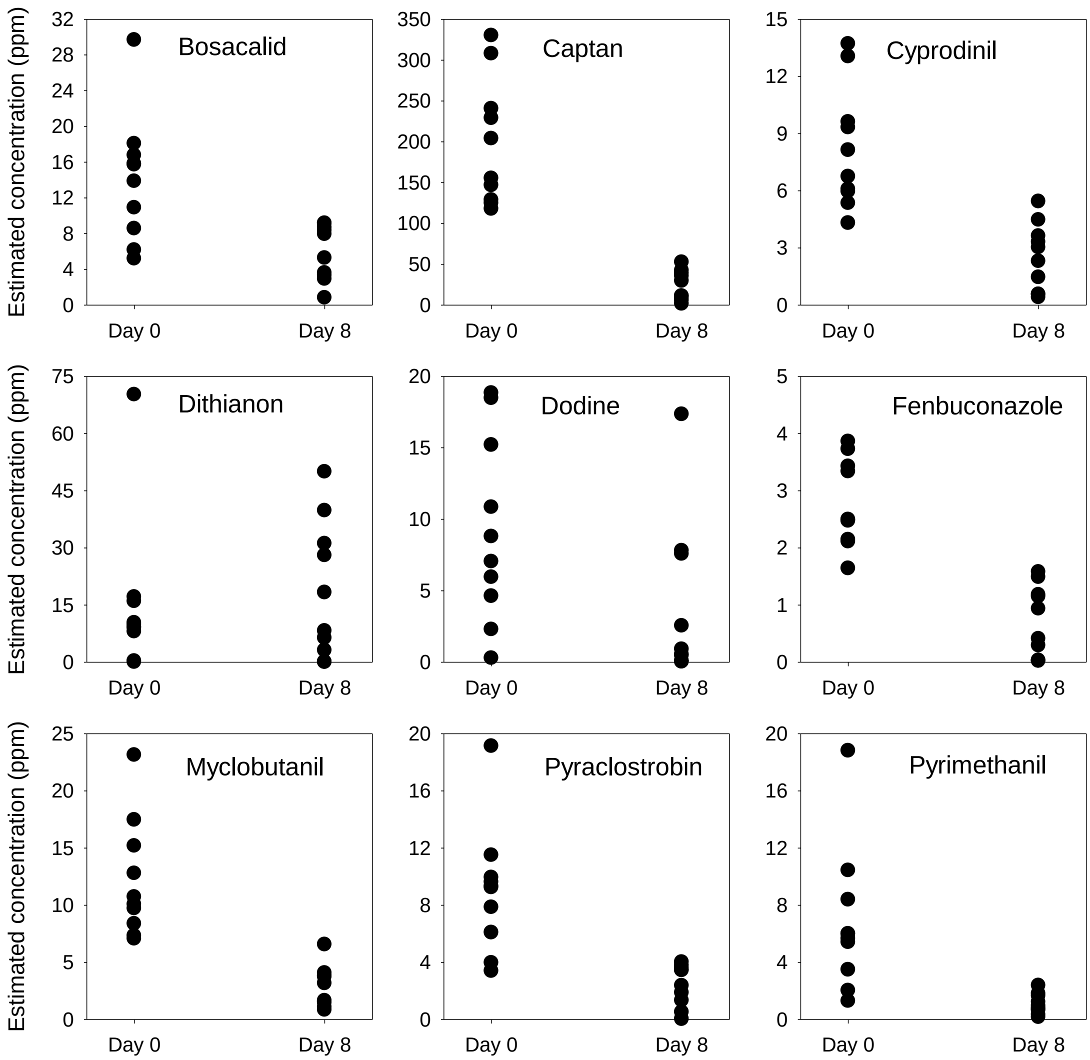

Figure 3. Estimated concentrations of active substances on the day of and eight days after the application in 2010 assuming 0.04 ml water wet 1 cm^2 of leaf surface

Cross-resistance among fungicides

As far as the reduced fungal sensitivity is concerned, we can only comment that, as expected, isolates from problematic orchards do tend to show an overall increase in their insensitivity to myclobutanil, compared to published baseline levels in several countries, including UK (Köller *et al.*, 1991; Braun & McRae, 1992; Sholberg & Haag, 1993; Jobin & Carisse, 2006, 2007). The reduced sensitivity to myclobutanil is consistent with the previous study conducted at EMR (Gao *et al.*, 2009a) and those published studied for other countries (Jones, 1981; Sholberg & Haag, 1993; Köller *et al.*, 1997; Jobin & Carisse, 2007).

Myclobutanil is the principal DMI fungicide used to control scab because of its additional control of powdery mildew. Overuse of myclobutanil has gradually reduced the fungal sensitivity to this fungicide, as demonstrated in the UK and in other countries. It is therefore appropriate to develop a control strategy that is less reliant on myclobutanil, particularly in those areas where this product has already been used frequently in the past. There are several alternative scab fungicides that could be deployed in certain strategies to manage scab whilst reducing the risk of the development of fungicide resistance. To develop and implement such an anti-resistance strategy based on integrated use of different fungicides, information on cross-resistance in *V. inaequalis* among these fungicides is needed.

The sensitivities to the two DMI fungicides (myclobutanil and fenbuconazole) were significantly correlated, confirming the previous report (Gao *et al.*, 2009b). However, it should be noted that the strength of the correlation may vary with the origin of the isolates as found in the previous study (Gao *et al.*, 2009b). Conversely, extensive use of fenbuconazole also led to reductions in the sensitivity of *Blumeriella jaapii* to myclobutanil (Proffer *et al.*, 2006). However, cross-resistance among DMI fungicides has been shown not to be universal. Positive cross-resistance is often observed among some DMI fungicides e.g. in *Cercospora beticola* (Karaoglanidis & Thanassoulopoulos, 2003), *Sclerotinia homoeocarpa* (Hsiang *et al.*, 1997) and *Cladosporium caryigenum* (Reynolds *et al.*, 1997), but not observed among some DMIs in other studies e.g. in *Cladosporium caryigenum* (McManus *et al.*, 1999) and *Mycosphaerella graminicola* (Mavroeidi & Shaw, 2005) or even negative cross-resistances among DMI fungicides in *Tapesia acuformis* (Leroux *et al.*, 2000). Thus, there are no consistent relationships in fungal responses to fungicides with similar control mechanisms, which indicate that it is necessary to assess such a relationship for relevant fungicides rather than inferred from previous studies on different pathogens.

The correlation in sensitivities to cyprodinil and pyrimethanil is expected as these two fungicides have the same model of action in disrupting methionine biosynthesis. However, the strong correlation in the sensitivity to dithianon with several other fungicides is unexpected since dithianon is believed to have multi-site contact activity. Further research is needed to confirm these correlations with isolates many different sources, including those baseline isolates.

Fungal sensitivities to dodine and captan were not significantly correlated with those to other fungicides. Although the sensitivity to boscalid was not correlated with those to other fungicides, the sensitivity to pyraclostrobin, the other active ingredient of Bellis, was significantly correlated with those to three other fungicides. Sensitivity to pyrimethanil was only correlated with that to cyprodinil. It should be noted that although there are significant correlations in the fungal sensitivities to some pairs of test fungicides, such correlation is far from perfect. Thus, broadly speaking, the eight fungicides may be divided into two groups: (1) Radspor (dodine), Scala (pyrimethanil) and Captan, and (2) the other five products. Fungicides from the first group may be used in mixtures with any other product, except Scala with Switch (cyprodinil). In contrast, mixed (or alternate) use of fungicides from the second group should need careful considerations [although their mixed or alternate use is not completely ruled out because of imperfect correlation].

It should be noted that actual disease severity depends on several other factors, in addition to fungal sensitivity to fungicides, including the absolute fungal growth rates under field conditions, spray coverage, inoculum strength and host growth.

Fungicide deposition

Fungicide residues immediately after the application are at a much higher concentration than the usual range of *in vitro* fungicide testing concentrations, and remain high even eight days after the application (during which there was no rainfall). However, the residues in 2009 declined rapidly following a rainfall event after application, except for dithianon for which no significant reduction was observed. Residues on some of sampled leaves that were rain-washed after fungicide application were less than the higher end of ED50 values of tested isolates for several chemicals, which may lead to successful scab infection. However, it should be noted that for most samples the residues were much larger than the higher end of ED50 values of tested isolates. Thus, failure to control scab was unlikely mainly due to the reduced sensitivity, other main factors may include lack of adequate coverage due to reduced volume, unprotected young tissue due to rapid host growth, and wash-off inoculum due to rain soon after fungicide application. In particular, it should be noted that the faster the growth rate, the more leaf area are not covered with previous fungicide applications on a given application interval.

Conclusions

- Sensitivities to captan, boscalid and dodine were not significantly correlated with those to other scab fungicides
- Sensitivity to pyrimethanil was only correlated with cyprodinil
- Sensitivity to the other fungicides (myclobutanil, pyraclostrobin, dithianon, fenbuconazole and pyraclostrobin are significantly positively correlated with those to at least three of these fungicides
- Mixed (or alternate) use of captan, dodine and pyrimethanil with other fungicides should be recommended over other combinations of fungicides
- The initial deposition concentration of fungicides on leaves, following application at the full recommended rate at 200 L/ha, is higher than the concentration at which reduced sensitivity was reported
- Heavy rainfall resulted in significant loss of all fungicides (except dithianon) on leaf surfaces
- The faster the growth rate, the more leaf area is not covered with an earlier fungicide application for any given application interval
- Thus, spray interval (and possibly the rate) needs to be adjusted according to the past/predicted rainfall and the rate of host growth

Technology transfer

Because of the sensitivity of this work, we purposely did not actively involve ourselves with technology transfer activities with growers until we have obtained clearer results.

- We discussed the project results with several consultants
- We held in-depth discussions of the work with BASF researchers and exchanged notes on protocols of *in vitro* and *in vivo* testing for sensitivities to fungicides in February 2010

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