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Variation for sensitivity of *Venturia inaequalis* to demethylation inhibiting (DMI) and strobilurin fungicides. Second and Final Report

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Background

A grant from APRC has helped to fund a visit by Dr Ezra Shabi (Volcani Center, Israel) to HRI Wellesbourne to contribute to research on variation for response to fungicides in *Venturia inaequalis* (causal agent of apple scab). Dr. Shabi worked at HRI from January to July 1997. This report is a continuation of the report (covering July - December 1996) submitted to APRC in April 1997. Part of this work was presented as a poster at the International Conference: "Resistance '97 - an Integrated Approach to Combating Resistance.", 14-16 April 1997, Rothamsted, UK. (Abstract appended).

Work has continued to concentrate on the variation between isolates of *V. inaequalis* from the UK and Israel for sensitivity to fungicides from the DMI group (eg. myclobutanil) and a new group of fungicides, the strobilurins represented by azoxystrobin (AS - Zeneca) and kresoxim methyl (KM - BASF).

The objectives of the work were as follows: 1) to determine if reduced sensitivity to DMI fungicides occurs in Israeli population of *V. inaequalis*; 2) to examine variation for sensitivity to a range of DMI fungicides among isolates of *V. inaequalis* from UK and Israel; 3) to develop methods for assaying variation for sensitivity to strobilurin fungicides among isolates of *V. inaequalis* and 4) to examine variation for sensitivity to strobilurin fungicides among isolates of *V. inaequalis* from UK and Israel known to vary in response to DMI fungicides.

Methods

In vivo assays

Mass spore populations or single spore isolates of *V. inaequalis* were tested for sensitivity to fungicides on treated apple seedlings. The DMI fungicides: myclobutanil, flusilazole (both triazoles) and pyrifenox (a member of the pyridine group) were assessed in post inoculation tests (fungicide applied 48h after inoculation). The concentration of fungicide used in these tests (50 µg/ml for myclobutanil and pyrifenox; 20 µg/ml for flusilazole) were similar to the rates currently in use for scab control in the UK and Israel (based on spray applications to run-off at about 1000 litre/hectare).

The strobilurin fungicide kresoxim methyl (KM) was assessed in pre-inoculation tests (fungicide applied 24h before inoculation) and the concentration of KM used ranged from 2.5 - 30 µg/ml. The rate used in the field for scab control in Israel is 75 µg/ml.

Assessment were made after inoculation of the numbers of seedlings exhibiting sporulation and the severity of infection as determined by leaf area covered by sporulation.

In vitro assays

DMI fungicides

Agar media amended with 0, 0.5 and 2.0 µg myclobutanil/ml were used and differences in fungal growth based on colony diameter recorded. Single spore isolates of cultures from several orchards were compared to the standard sensitive isolate UK-E1 and the reduced sensitive isolate UK L92/01.

Strobilurin analogue fungicides

Agar media amended with 0.0025 - 1.25 µg KM/ml or 0.005 - 2.5 µg AS/ml were used. Mass spore populations were assessed for their sensitivity by assessing incidence of germination and germ-tube length. This method was also used to assess the sensitivity of spore populations obtained from cultures and from scab lesions derived from single spore isolates. Sterile spore suspensions were prepared from cultures of single spore isolates. Ten µl drops of suspension were placed on the media and spore germination evaluated after 24 - 48h incubation at 16 - 18°C. Spore suspensions from cultures of single spore isolates were also used to inoculate apple seedlings. Scab lesions that subsequently developed from such inoculations were further multiplied on new stocks of seedlings.

Results

DMI fungicides

In vitro assays.

Agar media assays were used to compare the sensitivity to myclobutanil (0.5 µg/ml) of 145 single spore isolates from Israel with three UK and two Polish isolates exhibiting reduced sensitivity and a fully sensitive isolate, UK-E1. Of the 69 Israeli isolates sampled from seven Red Delicious (RD) orchards 66 were reduced sensitive and three were sensitive. Seventy six of the Israeli isolates were from three Anna orchards and of these 43 were sensitive. The remaining 33 exhibited reduced sensitivity and 28 of these came from a single Anna orchard at Be'er Tovia.

In vivo assays.

Apple seedlings were inoculated with spore suspensions prepared from infected leaves from 12 Israeli orchards (seven RD and five Anna). Scab lesions obtained from these mass spore populations were multiplied on seedlings and then compared with scab populations from the UK, two isolates from Poland and the standard sensitive isolate, UK-E1 (Table 6). ***Note that Tables are numbered in sequence with the first report.***

All five populations from Anna were as sensitive as the reference isolate UK-E1. Scab lesions obtained from a single spore isolate from an Anna orchard and classified as reduced sensitive *in vitro* was shown to be sensitive *in vivo*. Moreover, in the Anna orchard (Be'er Tovia) from which the reduced sensitive isolates had been isolated, myclobutanil treatments gave excellent control of scab (1.3% compared to 70.9% for the untreated controls; Table 10). The few scab lesions obtained from this myclobutanil plot were multiplied on apple seedlings and when tested on seedlings treated with myclobutanil (50 µg/ml), pyrifenox (50 µg/ml) and flusilazole (20 µg/ml) were found to be sensitive. Seven Israeli populations derived from RD orchards showed some reduced sensitivity to DMI fungicides as indicated by an equivalent incidence

and severity of scab on inoculated seedlings to that observed for isolates from the UK exhibiting reduced sensitivity.

Strobilurin analogue fungicides

In vitro assays

Agar media were used to compare the sensitivity to KM and AS of single spore isolates and mass spore populations obtained from scab lesions. A range of sensitivities to KM and AS was recorded for 42 UK and 42 Israeli isolates that had never been exposed to strobilurins; colony growth was less than that observed on unamended agar but never totally restricted. The sensitivity to KM and AS of two other Israeli isolates obtained from a plot treated with strobilurins for one year were in the same range.

The incidence of germination and germ-tube length of spores prepared from cultures of single spore isolates were found to be more appropriate measures of sensitivity than colony growth. Most of the isolates tested for sensitivity using this method were highly sensitive. A discriminatory concentration of 0.005 µg KM/ml (or 0.03 µgAS/ml) would prevent germination of the majority of spores obtained from most of the isolates tested. A few single spore isolates derived from a RD orchard at Or Tal in Israel were less sensitive and were able to germinate, grow and sporulate on 0.02 µg KM/ml. or 0.12 µg AS/ml. Spores derived from one of the least sensitive of these isolates, SP1, were able to germinate on KM at 0.04 µg/ml or AS at 0.24 µg/ml. For most of the isolates tested it was noticeable that a few spores were germinating on higher concentrations of fungicide than the discriminatory concentration. Such spores were able to grow and sporulate after 4 - 6 weeks at these higher concentrations.

Most of the spores obtained from scab lesions from either the Or Tal orchard or from single spore isolates were also sensitive and did not germinate on KM at 0.005 µg/ml or AS at 0.03 µg/ml. Spore suspensions prepared from scab lesions arising from a few single spores that had originated from the Or Tal orchard were found to be less sensitive. The germination of several single spore isolates and mass spore populations on media amended with KM or AS are presented in Table 7 with the *in vivo* results in Tables 8 and Table 9.

Conclusions

DMI fungicides

Five mass spore populations of *V. inaequalis* from the UK and three mass spore populations from Israeli RD orchards were able to infect apple seedlings treated with myclobutanil (50 µg/ml), flusilazole (20 µg/ml) and pyrifenox (50 mg/ml). Nevertheless, the amount of scab that developed on the treated plants was lower than on untreated seedlings in most tests. In two tests, on seedlings treated 24h prior to inoculation with pyrifenox, UK D 94/08 and IL 11 exhibited a higher mean disease index (MDI) than on the untreated control plants. Four other Israeli mass spore populations from RD orchards sporulated on seedlings treated with myclobutanil 24 h before inoculation (Table 6). Two isolates from Poland and two Israeli single spore isolates from RD orchards also sporulated on seedlings treated with DMI fungicides.

The 48h post-inoculation application of DMI fungicides to seedlings was considered to be a more appropriate method for sensitivity assay than pre-inoculation application of fungicide . By applying the fungicides after the infection process was completed (48h. in 16-18°C in a wet chamber) the possibility of 'escapes' was reduced compared to the pre-inoculation method

where some young, unfolded leaves (which are the most susceptible leaves to scab) would not be covered adequately with the fungicide. The post-inoculation spray assay is suitable for DMI fungicides since these systemic fungicides have eradicant activity but is not suitable for the evaluation of protectant fungicides. Although the Anna orchards in Israel have been treated with DMI fungicides for more than 15 years the amount of scab seen on DMI treated seedlings inoculated with isolates and mass spore populations derived from these orchards was negligible. Four populations and two single spore isolates from Anna behaved similarly to the standard sensitive isolate UK-E1. The *in vivo* results do not however correlate with the *in vitro* tests of single spore isolates derived from Anna. Of 76 single spore isolates derived from Anna orchards, 43 were sensitive and 33 exhibited reduced sensitivity in *in vitro* assays in comparison with standard isolates from the UK. Moreover, 27 of 30 single spore isolates from the Anna orchard at Be'er Tovia exhibited reduced sensitivity *in vitro* but tests, *in vivo* (2 pre and 3 post inoculation), of populations derived from IL 6 had shown these to be sensitive. SP 16, a single spore isolate from Be'er Tovia, was found to be reduced sensitive *in vitro* but did not infect seedlings treated with DMI fungicides. In tests conducted in this orchard over four seasons (1994 - 1997) myclobutanil was very effective in controlling scab.

Our results indicate that the sexual stage of V. *inaequalis* could play an important role in the development of resistance to DMI fungicides. The perithecial stage is observed in the UK and in the regions of Israel where there are RD orchards but not in the region where Anna is grown. Variation created through the sexual cycle, on which selection can act, could result in a more rapid development of populations exhibiting reduced sensitivity. It is suggested that the monitoring of *V. inaequalis* populations from the UK and Israel for sensitivity to DMI fungicides should become an important component of scab management. *In vivo* tests are essential for accurate decision making in this respect.

Strobilurin analogue fungicides

Scab lesions that developed on seedlings treated with low concentrations of KM indicate a potential risk of resistance in *V. inaequalis* to strobilurin analogue fungicides. Although scab developed on seedlings treated with 7.5 - 30 µg/ml KM and the concentration currently used for control of scab in the orchard is 75 µg KM/ml (Table 10) the risk of resistance occurring should be considered. Strobilurin analogue fungicides are being introduced for the control of a wide range of fungal pathogens including apple scab and powdery mildew. The selection pressure toward insensitivity could increase following commercial use of these fungicides.

In *in vitro* tests, a few spores were found that behaved differently from the majority of spores on media amended with KM or AS. Even at the highest KM (1.25 µg/ml) or AS (2.5 µg/ml) concentrations, a few spores germinated and developed sporulating colonies. Some of the single spore isolates were reisolated from KM at 1.25 µg/ml and successfully inoculated onto apple seedlings to produce a source of inoculum. For example, SP1 was isolated from a RD orchard in Israel in 1994 before strobilurins had been introduced into the area. SP131 is a single spore isolate taken from a sporulating colony of SP1 growing on KM at 1.25 µg/ml. This isolate was subcultured onto KM at 1.25 µg/ml. Scab lesions that developed after inoculation of seedlings with SP131 were tested *in vitro* against AS at 0.8µg/ml. SP151 was a single spore isolate derived from one of the few spores that germinated in this test and this has been subcultured on KM amended media and used subsequently in *in vitro* tests and to inoculate apple seedlings.

Although there is little direct evidence to suggest that DMIs are failing to control scab in the UK, isolates of *V. inaequalis* that show reduced sensitivity to these chemicals can be found. Strobilurin analogue fungicides are effective in controlling apple scab at low concentrations and

are already being introduced commercially into some EC countries; development of KM in the UK is likely in the future. Monitoring of the *V. inaequalis* population for sensitivity to strobilurins should continue before the introduction of these fungicides to the UK. The results presented in this report, and from any further monitoring studies, about the variation in sensitivity to strobilurins within *V. inaequalis* population should inform future scab management strategies in the UK. The strobilurin fungicides could replace or be used as a partner with the DMIs if myclobutanil, flusilazole and pyrifenox fail to control scab in the future. Use of strobilurins, that have a different mode of action from the DMIs, is likely to reduce the rate with which isolates insensitive to DMI fungicides might be selected and thereby maintain durable control.

Summary

- *In vitro* and *in vivo* assays have been developed for determining the relative sensitivity of isolates of *V. inaequalis* to strobilurin fungicides; an *in vivo* assay is preferred.
- There is no evidence for any association between reduced sensitivity to DMI fungicides and reduced sensitivity to strobilurin fungicides.
- DMI fungicides behave similarly in respect of isolates of *V. inaequalis* exhibiting reduced sensitivity.
- Some evidence was obtained for heterogeneity of spore populations with respect to strobilurin sensitivity on which selection could act.

Table 6 Summary of *in vivo* tests for variation in sensitivity to DMI fungicides among isolates of *Venturia inaequalis* from numerous sources using seedlings treated pre (24h) or post (48h) inoculation

Isolate	Cultivar	*In vitro* Classification	Number of tests		Mean disease index					
			Pre	Post	Pre			Post		
					Myc	Flu	Pyr	Myc	Flu	Pyr
UK isolates										
E1	Edward V11	Sensitive	7	7	0	0	10	0	0	3
L 92/01	Golden Delicious	Intermediate	7	6	23	11	46	13	3	40
D 91/117	Crispin	Sensitive	2	1	9	21	35	11	3	4
D 94/06	Tydemans Worcester	Sensitive	1	0	0	2	14			
D 94/08	Unknown	Untested	1	1	60	27	139	32	31	29
X 94/41	Cox	Intermediate	1	0	8	6	6			
X 94/77	*M. hopa*	Untested	1	0	55	54	58			
Polish isolates										
Pol-1	Unknown	Reduced Sensitive	0	1				28	0	17
Pol-2	Unknown	Reduced Sensitive	2	2	26	11	52	43	10	55
Israeli isolates										
IL 1	Red Delicious	Untested	2	2	17			13	2	11
IL 2	Red Delicious	Untested	"1"	2	*			16	8	5
IL 11	Red Delicious	Untested	2	3	23	14	119	30	3	7
IL 9	Red Delicious	Untested	"1"	0	*					
IL 10	Red Delicious	Untested	"1"	0	*					
IL 12	Red Delicious	Untested	"1"	0	*					
AB 11-1	Red Delicious	Untested	1	3	15	3	23	2	1	8
SP 34	Red Delicious	Untested	1	0	14	0	22			
IL 6	Anna	Sensitive	2	3	3			0	0	0
SP 16	Anna	Reduced Sensitive	1	1	0	2	5			0
IL 4	Anna	Sensitive	1	0	0					
SP 4	Anna	Sensitive	1	0	0					
IL 20	Anna	Sensitive	2	0	0	0	0			
IL 25	Anna	Sensitive	0	1						0
IL 27	Anna	Sensitive	0	1						0

Key. "1" = Mean % cover was not recorded, only the number of infected seedlings.
* = Diseased seedlings noted but not quantified.
Myc = Myclobutanil @ 50 µg/ml, Flu = Flusilazole @ 20 µg/ml & Pyr = Pyrifenox @ 50 µg/ml

Table 7. *In vitro* variation for sensitivity of single spore isolates and populations of *V. inaequalis* not previously exposed to strobilurins

	% of control										
	Kresoxim methyl (µg/ml)						*Azoxy strobin* (µg/ml)				
Isolate	**0.0025**	**0.005**	**0.01**	**0.02**	**0.04**	**0.08**	**0.015**	**0.03**	**0.06**	**0.12**	**0.24**
UK isolates											
UK-E1	100	0	0	0	0	0	2	0	0	0	0
UK L 92/01[b]	50	0	0	0	0	0	1	0	0	0	0
UK-TR 8/ 51	100	20	10								
Israeli isolates											
OT 94-SP1	100	100	100	100	10	0	100	100	100	100	50
OT 96-2	100	100	100	50	0	0	100	100	100	40	4
OT 96-4	100	100	100	50	0	0	100	100	80	60	10
OT 94-SP2	100	100	100	0	0	0	100	100	60	6	0
OT 96-3	100	100	80	0	0	0	100	60	30	20	3
AB 96-11	100	50	10	2	0	0	60		20	0	0
IL 11[b]	100	50	10	0	0	0	60	20	2	0	0
IL 2[b]	100	30	6	0	0	0					
IL-6-97[b]		100	10	0							
IL 1[b]	100	100	50	10							
D 117[b]	100	100	50	10							
MS-1-1	100	20	0								
IL 20[b]	100	10	0								
SP 5		0	0								
SP 8		25	0								
SP 13		20	10	0							
SP 17		20	10	0							
SP 19		0	0	0							
SP 44		5	0	0							
SP 20		20	10	0							
IL 25[b]		100	0								
Polish isolates											
Pol-1		100	100	10							
Pol-2	100	6	5	0	0	0	30	10	0	0	0

Values are combined for spore germination and germ-tube length where 100 is equivalent to the unamended control .
b = Orchard population

Table 8. Summary of *in vivo* assays for sensitivity to Kresoxim methyl
Seedlings were treated 24 h before inoculation with 2.5, 7.5, 15, or 30 µg KM/ml.
of the source of inoculum.
S = Scab lesions. N = No infection. Superscript figures represent the previous fungicide treatment
ut = untreated.

Isolate	Kresoxim methyl concentration																			
	2.5	7.5						15							30					
Israeli isolates																				
SP1	S^{ut}	S^{ut}	$S^{7.5}$	$S^{7.5}$				$S^{7.5}$	$S^{7.5}$	$N^{7.5}$					$N^{7.5}$					
SP131	S^{ut}	S^{ut}	$S^{7.5}$	S^{15}	$S^{7.5}$	S^{15}	S^{15}	N^{30}	$S^{7.5}$	S^{15}	$S^{7.5}$	S^{15}	S^{15}	N^{30}	S^{15}	S^{15}	N^{15}	N^{30}	S^{15}	
SP132	S^{ut}	S^{ut}	$S^{7.5}$	$S^{7.5}$	$S^{7.5}$	$S^{7.5}$	$S^{7.5}$		$N^{7.5}$	$N^{7.5}$	$N^{7.5}$	$N^{7.5}$	$S^{7.5}$			$N^{7.5}$	$N^{7.5}$			
SP133	S^{ut}	S^{ut}	S^{ut}	$S^{7.5}$					$S^{7.5}$							$S^{7.5}$				
SP151		S^{ut}							S^{ut}	S^{30}	S^{30}					S^{ut}	N^{30}	N^{30}		
SP231	S^{ut}	S^{ut}	$S^{7.5}$						$S^{7.5}$							$N^{7.5}$				
OT 21	S^{ut}	S^{ut}	$S^{7.5}$	$S^{7.5}$					$N^{7.5}$	$S^{7.5}$										
OT 41	S^{ut}	S^{ut}	$S^{7.5}$	$S^{7.5}$	$S^{7.5}$				$N^{7.5}$	$S^{7.5}$	N^{30}					$S^{7.5}$	N^{30}			
AB 11-1		S^{ut}	$S^{7.5}$						N^{ut}	$S^{7.5}$	N^{30}					$S^{7.5}$	N^{30}			
MS 2-1-1		S^{ut}																		
SP5		S^{ut}							$N^{7.5}$							$N^{7.5}$				
SP13		S^{ut}							$S^{7.5}$							$N^{7.5}$				
SP19		S^{ut}																		
SP44		S^{ut}							$N^{7.5}$							$N^{7.5}$				
SP16		S^{ut}							N^{ut}	$N^{7.5}$						N^{ut}	$N^{7.5}$			
IL6-97-KM11		N^{ut}							N^{ut}							N^{ut}				
IL6-97-KM		S^{ut}							N^{ut}							N^{ut}				
IL6-97-KM-Mix		S^{ut}							S^{ut}	$S^{7.5}$						N^{ut}	$N^{7.5}$			
IL6-97-Myc.		S^{ut}							S^{ut}	N^{30}						S	N^{30}			
IL 11 (RD)		S^{ut}							S^{ut}							N^{ut}				
IL 25 (Anna)		N^{ut}							N^{ut}							N^{ut}				
IL 27 (Anna)		N^{ut}							N^{ut}							N^{ut}				
UK isolates																				
UK E1		S^{ut}							N^{ut}											
UK TR 50		S^{ut}							N^{ut}	$N^{7.5}$						$N^{7.5}$				
Polish isolates																				
Pol-1		S^{ut}	$S^{7.5}$						$S^{7.5}$	N^{15}	$N^{7.5}$					$S^{7.5}$	N^{15}	$N^{7.5}$		
Pol-2		S^{ut}							N^{ut}							N^{ut}				

IL 1, IL 2, IL 9, IL 10, IL 11, IL 12 (ex. Red Delicious); IL 4, IL 6, IL 20 (ex. Anna);
UK E1, UK L 92/01 have been tested in a total of four tests against kresoxim methyl @ 5 & 10 µg/ml and azoxystrobin @ 5 & 10 µg/ml. No scab resulted.

Table 9. Variation among single spore isolates and populations of *V. inaequalis* from Israel, UK and Poland for sensitivity to kresoxim methyl in an *in vivo* assay.
Fungicide applied 24h before inoculation.

	Concentration			
Isolate	Untreated	7.5	15	30
[b]SP 151	100	12.1	14.3	7.0
[b]SP 131-12300	100	23.8	37.7	10.4
[b]Pol-1	100	89.3	3.0	NT
SP 16	100	36.8	0.0	0.0
IL6 97 KM	100	3.1	0.0	0.0
IL6 97 Mix	100	83.0	7.0	0.0
IL 11	100	2.0	2.0	0.0
IL 25	100	0.0	0.0	0.0
Pol-2	100	17.0	0.0	0.0

Values are of Disease index = mean % infected area per seedling based on three susceptible leaves per seedling expressed as a % of Untreated control.

[b] Isolates in this assay were tested at a different time and symptoms recorded 19 days after inoculation. The remainder were recorded 15 days after inoculation.

Table 10. Incidence of scab in a 12 year old Anna orchard at Be'er Tovia, Coastal Plain, Israel treated with myclobutanil and strobilurin analogue fungicides during Jan-April 1997

Fungicide treatment	Conc. (µg/ml)	% scab infection
Kresoxim methyl (KM)	75	1.55
[b]Azoxystrobin (AS)	125	2.37
Myclobutanil	62.5	1.33
[c]KM or Myclobutanil		0.06
[d]KM or Myclobutanil		0.11
Untreated Control		70.89

[a] Mean % leaf scab following nine spray applications to run-off using a hand lance.

[b] Spray No 1, myclobutanil, sprays 2-9 AS.

[c] Sprays 1,2,5,6,9 KM (before infection periods); sprays 3,4,7,8 myclobutanil (following infection periods).

[d] Sprays 1,2,3,5,6,8,9 KM; sprays 4 &7 myclobutanil.

Variation in sensitivity of *Venturia inaequalis* to DMI and strobilurin fungicides

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Venturia inaequalis (scab) can cause heavy infections of fruit and leaves of apple. In Israel it is prevalent in two regions: 1) in orchards of the coastal plain and internal valleys, planted with cv Anna; and 2) in orchards of the Golan region planted with introduced cultivars such as Red Delicious (RD).

Leaves of RD that over-winter on the floor of the orchard produce perithecia routinely and ascospores serve as the primary inoculum every spring in the Golan region. In contrast, ascospores of *V. inaequalis* were not detected in leaves of Anna that over-winter on the floor of the orchards of the coastal plain. In Anna orchards, leaves infected with scab from the previous autumn and early winter (October through to January), and still hanging on the trees, serve as the source of conidial inoculum causing primary scab infections of the newly emerging buds. Frequent fungicide spray treatments are required to avoid heavy crop losses.During the early 1980s, DMI fungicides were introduced for the control of apple scab. DMIs remain the main class of fungicide for the control of apple scab and powdery mildew; consequently the pathogen population has been and continues to be widely exposed to these fungicides.

Single spore isolates and scab samples from the two regions in Israel have been compared with samples from the UK including isolate E1, obtained in 1949 and therefore not exposed to DMI fungicides. Most of the isolates from Israel that showed reduced sensitivity to DMIs were from the Red Delicious orchards in the Golan Heights; only one such isolate was obtained from an Anna orchard. This isolate was less sensitive *in vitro* but sensitive *in vivo*. Isolates with reduced sensitivity to DMI fungicides have also been found in the UK. Isolates with reduced sensitivity to myclobutanil were also found to be less sensitive to other DMI fungicides in both seedling and on agar media tests.

Two strobilurin analogue fungicides, a new class of fungicides which inhibits the cell respiration of fungi, azoxystrobin (AS) and kresoxim methyl (KM) have already been tested in Israel and found to be very effective for control of apple scab. The sensitivity of single spore isolates were tested on media amended with AS or KM. These *in vitro* tests showed a range of sensitivities to strobilurins among Israeli and UK isolates. Seedlings inoculated with single spore isolates or populations of *V. inaequalis* and tested with AS or KM are being used to forecast the risk of resistance to the strobilurins.