Project title:	Variation for sensitivity of *Venturia inaequalis* to demethylation inhibiting (DMI) and strobilurin fungicides
Project number:	TF 103 [Previously APRC SP 103]
Report:	Interim report 1997
Project leader:	Professor Ian Crute, HRI East Malling
Key words:	fungicide, strobilurin, demethylation inhibiting (DMI), *Venturia enaequalis,* apple scab, apple

This project report was originally issued by the Apple & Pear Research Council, under project number SP 103.

Report to APRC - Project SP 103 (Shabi, Roberts and Crute)
14 April 1997

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

E. Shabi[1], A.L. Roberts[2] & I.R. Crute[2]

[1]Department of Plant Pathology ARO, The Volcani Center, Bet Dagan 50250, Israel
[2]The Director's Group, Horticultural Research International, Wellesbourne, Warwick, UK.

Background

A grant from APRC has helped to fund a visit by Dr Ezra Shabi to HRI Wellesbourne to contribute to research on variation for response to fungicides in *Venturia inaequalis* (causal agent of apple scab). Apple scab can lead to heavy infections of fruit and leaves of apple in Israel and the UK and frequent fungicide spray treatments are required to avoid crop losses. Dr. Shabi arrived from Israel at the beginning of July 1996 and completed a 6 month period of work in December 1996. This collaboration was initiated at a conference on fungicide resistance held at the University of Reading in 1994 and reflects a common interest in assessment of the potential risk of loss of field control of scab due to the increase in isolates of *V. inaequalis* with reduced sensitivity to DMI fungicides currently used in spray programmes for scab control. Dr Shabi plans to continue the research during the period from mid January 1997 to July 1997. This report covers the first 6 months of the work. Effort has been concentrated on the variation between isolates of *V. inaequalis* from the UK and Israel to fungicides from the DMI group (eg. myclobutanil) and a new group of fungicides, the strobilurins. These are analogues of a natural product from the fungus *Strobilurus tenacellus* and inhibit the cell respiration of various fungi including *V. inaequalis* whereas the DMIs interfere with the biosynthesis of sterols, essential components of cell membranes.

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.

Apple scab in Israel

In Israel, apple scab 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 cultivars, susceptible to scab, 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. During the early 1980s DMI fungicides were introduced and 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.

Apple scab in the UK

Apple scab in the UK is routinely controlled by the use of fungicides including myclobutanil, a DMI fungicide. Isolates with reduced sensitivity to myclobutanil have been found in the UK. Over 50 samples of *V. inaequalis* from commercial plantings in which myclobutanil has been used have been examined for variation in sensitivity to the fungicide as part of an ongoing study. The majority of these samples when tested in an *in vitro* assay based on germling growth were found to be as sensitive to myclobutanil as a standard isolate obtained in 1949. However, samples from a site where scab control was considered to be inadequate were found to have a response to myclobutanil equivalent to a reference isolate exhibiting reduced sensitivity. The same assay has also been used to test the sensitivity of over 150 single lesion samples of *V. inaequalis* from both myclobutanil treated and untreated trees in an experimental plot at East Malling. The majority of samples that exhibited reduced sensitivity to myclobutanil were from fungicide treated trees although a substantial number were also obtained from untreated trees.

Methods

Apple seedlings and rootstocks have been used for *in vivo* assays in which populations or single spore isolates of *V. inaequalis* have been tested on plants treated with DMI or strobilurin fungicides before or after inoculation. Seedlings have been mainly used for these assays because although rootstocks are more suitable due to their uniformity of response, some difficulties were encountered in maintaining the environmental conditions necessary for successful infection by the fungus.

Single spore isolates have been tested *in vitro* using agar media containing myclobutanil or the strobilurin fungicides: azoxystrobin (AS) or kresoxim methyl (KM). The sensitivity of *V. inaequalis* to myclobutanil was compared *in vitro* and *in vivo* to other DMI fungicides which are currently in use for scab control in Israel and the UK. The DMI fungicides used in these assays were; difenconazole, fenbuconazole, flusilazole, myclobutanil, penconazole and pyrifenox.

In vitro assays, based on spore germination or mycelial growth, were compared for their suitability for the assessment of the sensitivity of *V. inaequalis* isolates to strobilurin fungicides.

Results

Agar media assays were used to compare the sensitivity to myclobutanil (at 0.50 µg/ml) of 69 Israeli single spore isolates (49 derived from seven RD orchards and 20 from three Anna orchards) with three UK and two Polish isolates exhibiting reduced sensitivity and the sensitive isolate UK-E1. Forty six of the RD isolates and one of the Anna isolates exhibited reduced sensitivity but this latter isolate was later shown to be sensitive *in vivo*.

Six Israeli populations derived from RD orchards showed reduced sensitivity to myclobutanil equivalent to a reduced sensitive population from the UK when tested in seedling assays. Three populations from Anna orchards were as sensitive as the reference isolate UK-E1. Examples are presented in Table 1. Isolates with reduced sensitivity to myclobutanil were also found to be less sensitive to other DMI fungicides in both seedling and agar media tests (Tables 2, 3 and 4). This is the first data to be obtained on cross-insensitivity for isolates for *V. inaequalis* from the UK.

Two strobilurin fungicides, azoxystrobin (AS) and kresoxim methyl (KM) have already been tested in Israel and found to be very effective for apple scab control in field experiments (125 µg/ml for AS and 75 µml for KM). As with any newly introduced fungicide, the selection pressure towards insensitivity is initially low until significant usage follows commercialisation. Isolates used in tests for sensitivity to DMI fungicides in plant and agar plate assays are now being examined to assess their sensitivity to strobilurins. Results so far indicate that spore germination is a sensitive and accurate method of *in vitro* assay for the strobilurins. A range of sensitivities to AS and KM was recorded for 42 UK and 42 Israeli single spore isolates never exposed to strobilurins when tested on agar media. The sensitivity of two other Israeli isolates obtained from a plot treated with strobilurins for 1 year were in the same range. The application of AS or KM to seedlings inoculated with single spore isolates or populations of *V. inaequalis* controlled the disease (Table 5).

Summary

- Single spore isolates and mass spore populations with reduced sensitivity to DMI fungicides have been found in the UK and Israel.
- Reduced sensitivity to DMI fungicides in Israel was found only from Red Delicious orchards where the pathogen population undergoes the sexual stage whereas the population from Anna orchards exposed to DMI fungicides since the early 1980s was sensitive.
- KM and AS at 10 µg/ml effectively controlled scab in seedling assays.
- Inhibition of spore germination was more sensitive than mycelial growth for *in vitro* assays of sensitivity to strobilurin analogue fungicides.

Table 1.
***In vivo* response to myclobutanil of nine isolates of *V. inaequalis* from Israel compared to two standard isolates from the UK.**

Isolate	Source	Treatment	Total seedlings	Infected seedlings	% Infected	% of Untreated
E1	UK	Untreated	12	10	83	
		Myclobutanil	12	0	0	0
L 92/01	UK	Untreated	13	13	100	
		Myclobutanil	17	14	82	82
IL 4	Israel/Anna	Untreated	13	10	77	
		Myclobutanil	15	0	0	0
IL 6	Israel/Anna	Untreated	8	7	88	
		Myclobutanil	12	1	8	9
IL 20	Israel/Anna	Untreated	19	14	74	
		Myclobutanil	12	0	0	0
IL 1	Israel/RD	Untreated	12	12	100	
		Myclobutanil	12	5	42	42
IL 2	Israel/RD	Untreated	14	12	86	
		Myclobutanil	17	10	59	69
IL 9	Israel/RD	Untreated	12	9	75	
		Myclobutanil	16	15	94	125
IL 10	Israel/RD	Untreated	15	13	87	
		Myclobutanil	8	6	75	86
IL 11	Israel/RD	Untreated	13	12	92	
		Myclobutanil	13	10	77	84
IL 12	Israel/RD	Untreated	11	9	82	
		Myclobutanil	11	5	45	55

Footnote: Two week old apple seedlings were treated with myclobutanil (50 µg/ml) 24 h before inoculation with *V. inaequalis*. Scab symptoms were recorded 14 d after inoculation.
Isolates E1, IL 4, IL 6 and IL 20 are considered sensitive while all other isolates exhibited reduced sensitivity

Table 2.
In vivo response to three DMI fungicides of isolates of *V. inaequalis* from Israel, Poland and UK.
Fungicide applied 24 h before inoculation and symptoms recorded 14 d after inoculation.

Isolate	Treatment (µg/ml)	Total seedlings	Infected seedlings	Mean % area of sporulation	Disease index
UK-E1	Untreated	13	10	29	100
	Myclobutanil @ 50	12	0	0	0
	Flusilazole @ 20	14	0	0	0
	Pyrifenox @ 50	14	6	2	7
UK-L 92/1	Untreated	18	16	33	100
	Myclobutanil @ 50	15	5	5	15
	Flusilazole @ 20	16	5	3	9
	Pyrifenox @ 50	15	12	10	30
Poland	Untreated	12	11	50	100
	Myclobutanil @ 50	13	9	18	36
	Flusilazole @ 20	13	8	5	10
	Pyrifenox @ 50	13	8	24	48
IL Anna (SP 16)	Untreated	16	13	40	100
	Myclobutanil @ 50	16	0	0	0
	Flusilazole @ 20	17	1	1	3
	Pyrifenox @ 50	13	4	3	8
IL RD (SP 34)	Untreated	15	11	22	100
	Myclobutanil @ 50	15	6	3	14
	Flusilazole @ 20	16	0	0	0
	Pyrifenox @ 50	12	9	6	27

Footnote:
Mean % area of sporulation = Mean % area per leaf covered in sporulation based on two susceptible leaves per seedling.
Disease index = Mean % area of sporulation expressed as a % of the untreated control.

Table 3.
In vivo response to five DMI fungicides of six isolates of *V. inaequalis* from the UK.
Fungicide applied 24h before inoculation and symptoms recorded 18d after inoculation

Isolate	Treatment (µg/ml)	Total seedlings	Infected seedlings	Mean % area of sporulation	Disease index
L 92/01	Untreated	18	16	14	100
	Myclobutanil 50	19	5	1	7
	Fenbuconazole 50	18	3	<1	3
	Flusilazole 20	18	11	2	14
	Penconazole 50	19	12	2	14
	Pyrifenox 75	18	16	10	71
D 117	Untreated	19	18	13	100
	Myclobutanil 50	20	6	1	8
	Fenbuconazole 50	20	10	2	15
	Flusilazole 20	20	12	5	38
	Penconazole 50	17	6	2	15
	Pyrifenox 75	20	17	7	54
D 94/06	Untreated	19	18	21	100
	Myclobutanil 50	18	0	0	0
	Fenbuconazole 50	18	3	<1	2
	Flusilazole 20	20	4	<1	2
	Penconazole 50	18	5	2	10
	Pyrifenox 75	18	6	3	14
D 94/08	Untreated	20	16	7	100
	Myclobutanil 50	17	8	4	57
	Fenbuconazole 50	19	12	3	43
	Flusilazole 20	12	3	2	29
	Penconazole 50	17	8	3	43
	Pyrifenox 75	19	13	9	129
X 94/41	Untreated	17	17	27	100
	Myclobutanil 50	19	7	2	7
	Fenbuconazole 50	19	5	1	4
	Flusilazole 20	19	9	2	7
	Penconazole 50	18	8	3	11
	Pyrifenox 75	19	8	2	7
X 94/77	Untreated	20	15	9	100
	Myclobutanil 50	18	11	5	55
	Fenbuconazole 50	17	10	5	55
	Flusilazole 20	18	11	5	55
	Penconazole 50	17	14	8	89
	Pyrifenox 75	20	14	5	55

Footnote:
Mean % area of sporulation = Mean % area per leaf covered in sporulation based on two susceptible leaves per seedling.
Disease index = Mean % area of sporulation expressed as a % of the untreated control.

Table 4.
In vitro response to seven DMI fungicides of single spore isolates of V. inaequalis; seven from Israel, three from UK and one from Poland
(S = Sensitive, RS = Reduced sensitive).
Colony growth compared after 30 days incubation at 18°C

Fungicide Concentration µg/ml	Myclobutanil 0.5	Fenbuconazole 0.1	Penconazole 0.5	Hexaconazole 0.1	Flusilazole 0.1	Difenoconazole 0.1	Pyrifenox 0.5
Isolate							
IL Anna SP 4	S	S	S	S	S	S	S
IL Anna SP 23	S	S	S	S	S	S	S
IL Anna SP 16	RS	RS	RS	RS	RS	RS	S
UK D UK L UK X	RS	RS	RS	RS	RS	RS	RS
IL RD SP 37							
IL RD 96-MS	RS	RS	RS	RS	RS	RS	RS
IL RD 96-AB	RS	RS	RS	RS	RS	RS	RS
IL RD 96-OT							
Pol-2	RS	RS	RS	RS	RS	RS	RS

Table 5.
In vivo response to myclobutanil and two strobilurin fungicides, azoxystrobin (AS) and kresoxim methyl (KM) of two UK and two Israeli isolates of *V. inaequalis*.
Fungicide applied 24h before inoculation (Pre) or 48h after inoculation (Post).

Isolate	Treatment (µg/ml)	Experiment 1 Mean % area of sporulation	Disease index	Experiment 2 Mean % area of sporulation	Disease index
UK-E1	Untreated	24	100	39	100
	Myclobutanil 50 Pre	<1	<1	0	0
	Myclobutanil 50 Post	0	0		
	AS 10 Pre	1	4		
	KM 10 Pre	0	0		
	KM 10 Post	2	8		
UK-L92/01	Untreated	25	100	35	100
	Myclobutanil 50 Pre	12	48	12	34
	Myclobutanil 50 Post	10	40		
	AS 10 Pre	4	16	0	0
	KM 10 Pre	0	0	0	0
	KM 10 Post	6	24		
IL 1 RD	Untreated	25	100	30	100
	Myclobutanil 50 Pre	1	4	9	30
	Myclobutanil 50 Post	3	12		
	AS 10 Pre	3	12	3	10
	KM 10 Pre	0	0	0	0
	KM 10 Post	2	8		
IL 6 Anna	Untreated	24	100	27	100
	Myclobutanil 50 Pre	1	4	<1	2
	Myclobutanil 50 Post	0	0		
	AS 10 Pre	3	13	1	4
	KM 10 Pre	<1	<1	1	4
	KM 10 Post	1	4		

Footnote:
UK-E1 is a single spore isolate, UK-L 92/01 a mass spore sample for both experiments
Mean % area of sporulation = Mean % area per leaf covered in sporulation based on two susceptible leaves per seedling.
Disease index = Mean % area of sporulation expressed as a percentage of the untreated control.