SENSITIVITY OF *VENTURIA INAEQUALIS* (BLACK SPOT) ON APPLES TO DMI FUNGICIDES IN NEW ZEALAND

H.G. WHELAN, M.R. BUTCHER and R.E. GAUNT*

Plant Protection Research Unit, Lincoln University, Canterbury

**Department of Biochemistry and Microbiology, Lincoln University, Canterbury*

SUMMARY

Sensitivities (EC_{50} and EC_{95} values) of isolates of *Venturia inaequalis* to demethylation inhibiting (DMI) fungicides were determined. Isolates (271) were obtained from 54 commercial properties in the six main apple production areas in New Zealand. The majority of isolates were classified as sensitive. A few isolates (2.2%) had high $EC_{50/95}$ values, indicative of a shift towards reduced sensitivity. Three properties with a history of frequent DMI fungicide use had values high enough to be of concern for the future for resistance management. Some degree of cross resistance was shown to DMI fungicides not used on the properties concerned. Regional differences in sensitivity were mostly small. The detection of reduced sensitivity is discussed in relation to the possibility of spread, continued efficacy and recommendations for DMI use.

Keywords: *Venturia inaequalis*, DMI fungicides, reduced sensitivity, cross resistance.

INTRODUCTION

Demethylation inhibitor (DMI) fungicides have been used increasingly for effective control of apple black spot, caused by *Venturia inaequalis* (Cooke) Wint. All DMI fungicides have the same mode of action. Repeated applications of fungicides with a specific mode of action generally favours the rapid build-up of fungicide resistant strains and may lead to disease control failures (Ogawa *et al* 1983). Cross resistance may develop to other fungicides with the same mode of action.

The New Zealand Committee for Pesticide Resistance (NZCPR, Prince *et al* 1989) recommended that the number of DMI plus protectant fungicide applications in apple orchards should not exceed four per season, preferably confined to the period from tight cluster to second cover. In seasons of extreme disease pressure, a maximum of six applications as mixtures can be used, with increased risk of resistance.

Reduced sensitivity (four to eight fold) of *V. inaequalis* strains to DMI's has been reported in the USA and Germany (Stanis and Jones 1985). The objective of this study was to detect whether there have been changes in the sensitivity to DMI fungicides in *V. inaequalis* populations in New Zealand. *V. inaequalis* infected tissue was collected from properties with frequent DMI usage to increase the likelihood of detecting any such changes. This study did not determine the average level of resistance of *V. inaequalis* strains to DMI's in New Zealand orchards because properties with little or no DMI fungicide usage were not sampled.

MATERIALS AND METHODS

Infected leaves and fruit were sampled from 54 commercial properties in Auckland, Waikato, Hawkes Bay, Nelson, Canterbury and Central Otago. Properties were selected only if two or more DMI fungicides had been applied in each of the previous two seasons. Young leaves (95% of total sample) and fruit infected by *V. inaequalis* were collected no more than four weeks after the latest application of DMI fungicides. Sampling dates ranged from mid December 1990 (Auckland/Waikato) to late January 1991 (Canterbury). Triforine was not applied in any of the surveyed properties in 1989/90 or 1990/91. Less than 10% of properties applied fenarimol or triadimefon at least once in the 1990/91 season. Eighteen and 66% of growers applied penconazole or

Proc. 45th N.Z. Plant Protection Conf. 1992: 289-294

myclobutanil at least once in the 1990/91 season respectively. Myclobutanil was also commonly used in the previous season.

Conidia were scraped from newly sporulating lesions on unsterilized leaves or fruit, streaked onto plates of water agar (20 g /litre Davis) and incubated for 2 days at 18-20°C with a 12 photoperiod of fluorescent and incandescent light. Conidia which germinated were transferred aseptically to plates of potato dextrose agar (PDA; 39 g/litre Gibco), amended with benzylpenicillin (100 mg/litre) and streptomycin sulphate (100 mg/litre) to reduce bacterial growth, and incubated for 2 to 3 weeks at 18-20°C with a 12 photoperiod of fluorescent and incandescent light. Isolates were maintained on PDA slopes at 1°C. Two hundred and seventy one isolates of *V. inaequalis* were obtained and tested for fungicide resistance.

Fungicides and concentrations (mg/litre) chosen for resistance testing were:

Fenarimol (Rubigan FLO, Rhone-Poulenc (NZ) Ltd); 0.01, 0.02, 0.05, 0.15 and 0.4
 Myclobutanil (Systhane 40 W, Rohm and Haas New Zealand Ltd); 0.02, 0.05, 0.1, 0.2 and 0.6
 Penconazole (Topas 10WP, Ciba Geigy New Zealand Ltd); 0.01, 0.02, 0.04, 0.07 and 0.15
 Triadimefon (Bayleton 5DF, Bayer New Zealand Ltd); 0.1, 0.3, 1.0, 3.0 and 10
 Triforine (Saprol 190EC, Shell Chemicals (NZ) Ltd); 0.2, 1.0, 4.0, 20 and 40.

Fenarimol, penconazole and triadimefon were suspended in sterile water and triforine and myclobutanil were suspended in 95% ethanol and diluted to volume with sterile water. The final concentration of ethanol in myclobutanil and triforine amended media was 0.4%. Fungicide suspensions, benzylpenicillin (200 mg/litre) and streptomycin sulphate (200 mg/litre) were added to an autoclaved mixture of PDA (20 g/litre) and water agar (10 g/litre). Mycelial plugs (3 mm diameter) were taken from the colony margin of each isolate grown on PDA, and placed on two replicate plates of each fungicide treatment. The diameter of each colony was measured after 17-21 days (fenarimol, triadimefon, penconazole and myclobutanil) incubation at 18-20°C in darkness. Triforine was found in preliminary tests to be unstable when plates were incubated for long periods and thus the diameter of each colony was measured after 11 days incubation on triforine amended plates.

Sensitivity of *V. inaequalis* was expressed as EC_{50} and EC_{95} values from linear relationships between colony diameter (mm) and $\log_{10}$ concentration of fungicide. Sensitivity to each DMI was calculated at a national, regional and property level. Based on currently available information, isolates with reduced sensitivity to DMI fungicides were defined as having EC_{50} or EC_{95} values four times greater than the mean values for New Zealand.

RESULTS

Reduced sensitivity was found in only a small proportion of the sampled population. The proportion of isolates with reduced sensitivity (EC_{50}) to fenarimol, myclobutanil, penconazole and triadimefon was 0.4, 2.2, 1.5 and 1.1% respectively. The proportion of isolates with reduced sensitivity (EC_{95}) to fenarimol, myclobutanil, penconazole and triadimefon was 1.9, 1.9, 1.1 and 0.7% respectively. No isolates were found to have reduced sensitivity to triforine. More variation in mean national $EC_{50/95}$ values was found for myclobutanil compared to other DMI fungicides (Table 1 and 2).

TABLE 1: EC₅₀s (mg/litre) of DMI for *V. inaequalis* isolated from commercial apple orchards.

Region	Fungicide				
	fenarimol	myclobutanil	penconazole	triadimefon	triforine
Auckland/ Waikato	0.049(0.0274) ¹	0.12(0.068)	0.032(0.0246)	1.4(0.65)	8.8(4.62)
Hawkes Bay	0.050(0.0211)	0.10(0.051)	0.033(0.0091)	1.5(0.79)	8.2(3.22)
Nelson	0.038(0.0129)	0.18(0.177)	0.041(0.0714)	1.7(0.98)	8.1(3.45)
Canterbury					
A ²	0.045(0.0233)	0.26(0.295)	0.051(0.0420)	2.3(2.15)	10.5(6.08)
B	0.038(0.0190)	0.13(0.045)	0.036(0.0127)	1.5(0.52)	8.2(2.70)
Central	0.046(0.0300)	0.16(0.104)	0.039(0.0136)	1.4(0.55)	7.9(2.67)
Otago					
ALL REGIONS	0.045(0.0223)	0.16(0.173)	0.039(0.0244)	1.7(1.20)	8.6(4.22)

¹ Mean and standard deviation (in brackets).

² Sensitivity in Canterbury was expressed as the mean and standard deviation calculated from (A) all isolates tested, and (B) all isolates tested except those from two properties which had very high EC₅₀ values.

TABLE 2: EC₉₅s (mg/litre) of DMI fungicides for *V. inaequalis* isolated from commercial apple orchards.

Region	Fungicide				
	fenarimol	myclobutanil	penconazole	triadimefon	triforine
Auckland/ Waikato	0.150(0.1710) ¹	0.77(0.620)	0.115(0.1546)	4.9(1.87)	35.7(13.38)
Hawke's Bay	0.150(0.1202)	0.51(0.371)	0.101(0.0482)	5.2(2.34)	39.8(10.09)
Nelson	0.134(0.0788)	0.85(0.828)	0.123(0.0664)	4.7(2.77)	38.2(13.86)
Canterbury					
A ²	0.173(0.1585)	1.17(1.369)	0.155(0.0938)	6.6(6.86)	37.3(12.00)
B	0.106(0.0590)	0.65(0.234)	0.119(0.0440)	4.3(1.25)	34.7(11.00)
Central	0.151(0.0791)	0.86(0.674)	0.118(0.0494)	4.7(2.05)	41.2(20.74)
Otago					
ALL REGIONS	0.149(0.1256)	0.81(0.849)	0.121(0.0910)	5.2(3.65)	38.3(13.46)

¹ Mean and standard deviation (in brackets).

² Sensitivity in Canterbury was expressed as the mean and standard deviation calculated from (A) all isolates tested, and (B) all isolates tested except those from two properties which had very high EC₉₅ values.

Isolates from Canterbury had mean EC₅₀ values for myclobutanil, penconazole and triadimefon 63, 68 and 35% higher than the national mean respectively (Table 1). Isolates from Canterbury had mean EC₉₅ values for myclobutanil and triadimefon 44 and 27% higher than the national mean respectively (Table 2). The higher EC_{50/95} values were because of the inclusion in the analysis of two properties with high values (Properties B and C; Table 3). Four other properties which applied three or more DMI fungicides in each season had high EC_{50/95} values (Table 3). Isolates collected from Hawkes Bay had EC_{50/95} values for myclobutanil 38% lower than the national mean.

TABLE 3 : EC₅₀s and EC₉₅s (mg/litre) of DMI fungicides for selected properties in New Zealand.

Chemical	EC value	Mean (SD) ¹	Property ²					
			A	B	C	D	E	F
fenarimol	EC ₅₀	0.045(0.0157)	0.108	–	–	–	–	–
	EC ₉₅	0.149(0.098)	0.511	0.563	0.380	–	–	0.342
myclobutanil	EC ₅₀	0.16(0.152)	–	1.00	0.66	–	0.41	–
	EC ₉₅	0.81(0.664)	1.90	4.21	2.75	1.69	1.90	–
penconazole	EC ₅₀	0.039(0.0220)	0.077	0.175	–	–	–	–
	EC ₉₅	0.121(0.0642)	0.40	0.381	0.250	–	–	–
triadimefon	EC ₅₀	1.7(1.07)	–	8.3	3.9	–	–	–
	EC ₉₅	5.2(3.16)	–	25.9	–	–	–	–
triforine	EC ₅₀	8.6(3.11)	–	24.3	17.4	–	–	–
	EC ₉₅	38.3(7.23)	–	–	–	–	–	–

¹ A mean EC₅₀ and EC₉₅ value, derived from approximately 5 isolates, was calculated for each property. These values were then averaged to provide a national property mean and standard deviation (SD).

² Properties with $\geq$ twice the national property mean (twice the mean was chosen rather than four times (see Materials and Methods) because the variation in national property means was smaller than for all isolates).

Property location and DMI's applied in 1989/90 and 1990/91 seasons were:

*Property A = Waikato; approximately 9 flusilazol in both seasons

*Property B = Canterbury; 6 myclobutanil/6 myclobutanil

*Property C = Canterbury; 6 myclobutanil/6 myclobutanil

*Property D = Nelson; total of 5 penconazole and myclobutanil/3 penconazole + 1 myclobutanil

*Property E = Nelson; 1 flusilazol + 1 penconazole + 1 myclobutanil / 1 flusilazol + 2 myclobutanil

*Property F = Hawkes Bay; 3 myclobutanil + 1 penconazole/3 myclobutanil + 1 penconazole.

DISCUSSION

Most isolates of *V. inaequalis* in New Zealand were sensitive to the DMI's tested. However, the occurrence of a small number of isolates with high EC_{50/95} values suggests there has been a slight shift in the population towards reduced sensitivity to DMI fungicides. A shift in the *V. inaequalis* population in New Zealand has also been reported from the 1987/88 to the 1989/90 season by Fowler and Robertson (1992). Cross resistance with other DMI fungicides not applied in the orchard spray programme was detected in some isolates with reduced sensitivity. For example, isolates from Property B grew on high concentrations of fenarimol, triadimefon and penconazole, even though these chemicals have not been applied. Isolates appeared to be less sensitive to myclobutanil compared to the other fungicides tested, reflecting the major usage of myclobutanil in previous seasons. Some properties with high usage of DMI currently have high sensitivity to the DMI's tested. Isolates with reduced sensitivity may not have been sampled from these properties because of the small number of samples taken from each property (five per property), or sensitivity changes may not have developed yet.

Isolates with reduced sensitivity were generally associated with three properties which applied DMI's regularly (six or nine sprays per year for at least the previous two seasons). This frequency exceeds the recommended maximum and is at a level where there is an increased risk of resistance to DMI fungicides (Prince *et al* 1987). The appearance of resistance in field populations does not necessarily mean that disease control failures will occur. Failures will occur only if resistance strains become dominant in the population. These three properties currently have nil, to slight disease

control problems (i.e. <5% fruit infected at time of sampling), but levels of control in the future may be reduced with continued frequent use of DMI fungicide applications.

Sensitivity to fenarimol, triadimefon and penconazole of the *V. inaequalis* population in New Zealand was considerably greater than for isolates in overseas countries (Table 4). New Zealand isolates were less sensitive to myclobutanil than sensitive isolates in Canada, but were six times more sensitive than resistant isolates. Properties sampled overseas have applied DMI's frequently over many years, and this exposure to fungicides may have contributed to reduced fungicide sensitivity compared to isolates in New Zealand. However, a few properties in New Zealand (Properties A, B and C; Table 3) had mean EC₉₅ values which were approaching some of the values for resistant isolates in other countries. This suggests that the New Zealand *V. inaequalis* population is beginning to shift away from being fully sensitive to fungicides.

TABLE 4: Comparison of sensitivity (mg/litre) to DMI fungicides of *V. inaequalis* isolated from orchards in New Zealand and overseas.

Country	Source of Isolates ¹	Fungicide				
		fenarimol	myclobutanil	penconazole	triadimefon	triforine
Germany and USA:						
Stanis and Jones (1985)						
	MIC ²					
	sensitive	0.4	—	—	—	—
	resistant	3.0	—	—	—	—
France:						
Thind <i>et al</i> (1986)						
	LC ₉₀					
	sensitive	0.25	—	0.26	8.0	97
	resistant	3.9	—	2.7	42.2	100
Italy and The Netherlands:						
Fiaccadori <i>et al</i> (1987)						
	ED ₉₅					
	sensitive	0.22	—	0.24	—	—
	resistant	0.47	—	0.45	—	—
Canada:						
Hildebrand <i>et al</i> (1988)						
	MIC					
	sensitive	—	<0.2	<0.2	—	2.2
	resistant	—	4.8	1.8	—	>5.0
New Zealand:						
Fowler and Robertson (1992)						
	MIC					
	variable	0.4	—	0.1	7.5	20
present study						
	EC ₉₅					
	variable	0.15	0.81	0.12	5.2	38.3

¹ Sensitive or resistant isolates were generally sampled from orchards with no history of DMI fungicides, or a history of intensive DMI usage respectively.

² DMI sensitivity was estimated by determining the concentration at which 90% (LC₉₀), 95% (ED₉₅, EC₉₅) or 100% (MIC) of mycelial growth was inhibited.

ACKNOWLEDGEMENTS

The authors are grateful to the following: AGCARM for providing funding for this project and Bayer New Zealand Ltd, Ciba Geigy New Zealand Ltd, Rhone-Poulenc (NZ) Ltd, Rohm and Haas New Zealand Ltd and Shell Chemicals (NZ) Ltd for providing chemicals; Fruit Federation New Zealand Ltd for assistance in organisation

of the sampling and orchardists for allowing samples to be taken from their properties.

REFERENCES

- de Waard, M.A. and Fuchs, A., 1982. Resistance to ergosterol-biosynthesis inhibitors. II. Genetic and physiological aspects. pp. 87-100 *In: Fungicide Resistance in Crop Protection*, J. Dekker and S.G. Georgopoulos (Eds). Pudoc, Wageningen, The Netherlands. 265pp.
- Fiaccadori, R., Gielink, A.J. and Dekker, J., 1987. Sensitivity to inhibitors of sterol biosynthesis in isolates of *Venturia inaequalis* from Italian and Dutch orchards. *Neth. J. Plant Path.* 93: 285-287.
- Fowler, M. and Robertson, D.I., 1992. Sensitivity of *Venturia inaequalis* to demethylation inhibiting fungicides (DMIs). *The Orchardist of N.Z.* 65: (In press).
- Hildebrand, P.D., Lockhart, C.L., Newbery, R.J. and Ross, R.G., 1988. Resistance of *Venturia inaequalis* to bitertanol and other demethylation-inhibiting fungicides. *Can. J. Plant Path.* 10: 311-316.
- Ogawa, J.M., Manji, B.T., Heaton, C.R., Petrie, J. and Sonoda, R.M., 1983. Methods for detecting and monitoring the resistance of plant pathogens to chemicals, pp. 117-162. *In: Pest Resistance to Pesticides*. G.P. Georgioui and T. Saito (Eds.). Plenum Press, New York, USA. 809pp.
- Prince, R.W., Moore, M.S. and Wearing, C.H., 1989. The New Zealand Committee on Pesticide Resistance 1989 Summary. *Proc. 42nd N.Z. Weed and Pest Control Conf.*: 278-292.
- Stanis, V.F., and Jones, A.L., 1985. Reduced sensitivity to sterol-inhibiting fungicides in field isolates of *Venturia inaequalis*. *Phytopath.* 75: 1098-1101.
- Thind, T.S., Clerjeau, M. and Olivier, J.M., 1986. First observations on resistance in *Venturia inaequalis* and *Guignardia bidwellii* to ergosterol-biosynthesis inhibitors in France. *Proc. 2nd Brit. Crop Protect. Conf. – Pests and Diseases*: 491-498.