REDUCED SENSITIVITY TO DMI FUNGICIDES IN *MONILINIA FRUCTICOLA* AND THE EFFICACY OF DMI FUNGICIDES FOR BLOSSOM BLIGHT CONTROL

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ABSTRACT

Significant reductions in sensitivity to triforine (a demethylation inhibitor, DMI) were detected in *Monilinia fructicola* populations from seven of nine Hawkes Bay orchards sampled in 1992. The mean EC_{50} of a wild population obtained from unsprayed home garden sites throughout New Zealand in 1990 was 3.3 mg/litre triforine (range for individual isolates, 0.5 - 10.8 mg/litre). In contrast, the mean EC_{50} values for the orchard populations ranged from 4.5 to 7.9 mg/litre triforine (range for individual isolates, 0.4 - 28.1 mg/litre). Four additional orchards were surveyed in 1994 and the mean EC_{50} values of these orchard populations ranged from 5.7 to 7.6 mg/litre triforine (range for individual isolates, 2.2 - 17.3 mg/litre). Representative isolates with reduced triforine sensitivity were also significantly less sensitive to three other DMI fungicides, bitertanol, cyproconazole and flusilazol. Isolates with reduced triforine sensitivity blighted peach flowers as well as sensitive isolates but there was no loss of efficacy when triforine or cyproconazole treated peach flowers were challenged with these isolates. These results suggest that significant changes in sensitivity to triforine have occurred but there has not been a loss of disease control.

Keywords: *Monilinia fructicola*, stone fruit, DMI sensitivity, DMI efficacy, triforine

INTRODUCTION

In seasons favourable for disease development, brown rot of stone-fruit, caused by *Monilinia fructicola* (Wint.) Honey, has caused serious yield reductions. In order to prevent flower infection in the spring and fruit rot at harvest, repeated fungicide applications are required in New Zealand orchards. Several DMI fungicides (bitertanol, cyproconazole, difenoconazole, flusilazol, prochloraz and triforine) are registered for brown rot control in New Zealand, and current resistance management strategies recommend a maximum of three DMI applications in any one season (Prince *et al.* 1989). In 1990, three orchards in the Hawkes Bay region reported inadequate disease control following DMI applications. Two of these orchards were found to have isolates with significantly lower triforine sensitivity than wild-type populations (Elmer *et al.* 1992). However, the emergence of significantly reduced sensitivity in field populations of *M. fructicola*, does not mean that disease control failures will occur. This depends on the frequency of fungicide application and the biological characteristics of isolates with reduced sensitivity.

This paper reports on changes in sensitivity of *M. fructicola* to triforine and other DMI fungicides, the pathogenicity on peach flowers of *M. fructicola* isolates with reduced triforine sensitivity, and the effectiveness of DMI fungicides when challenged with these isolates.

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METHODS

Orchard survey and sensitivity tests

Several stone fruit orchards were selected from the Hawkes Bay area. Up to 50 fruit infected with *M. fructicola* were sampled randomly using a stratified sampling pattern from each of nine orchards in March 1992 and four orchards in March 1994. Up to 31 mass cultures of *M. fructicola* were obtained from infected fruit using standard isolation procedures, transferred to V8 juice agar, sealed and stored at 3 °C before bioassay tests.

A solution of 190 g/litre triforine (Saprol) was prepared in sterile distilled water and diluted with autoclaved potato dextrose agar (PDA) at 50-55°C. It was dispensed (20 ml) into plastic petri dishes (85 mm diameter) to give final concentrations of 0, 0.1, 0.3, 1, 3, 10 and 30 mg/litre triforine 1992 tests and 0, 0.3, 1, 3, 7, 18, 30 mg/litre triforine in 1994 tests. Mycelial plugs (6 mm diameter) from the margin of actively growing 6-10 day old cultures on PDA were placed mycelial surface down on the test medium for all sensitivity tests. Two colony diameters were measured at right angles to each other after incubation at 25°C (±0.5°C) in the absence of light for 4 days and the growth rate (mm/day) calculated.

The sensitivity of each isolate was expressed as an EC_{50} value calculated from a dose response curve of growth rate (mm/day) to $\log_{10}$ triforine concentration (mg/litre). The home garden population data (reproduced from Elmer *et al.* 1992) was included for comparative purposes and four standard sensitive isolates from this population were included as checks in all 1992 and 1994 tests. Analysis of variance was used to compare the mean EC_{50} of the orchard populations.

Cross-resistance tests

Four isolates with significantly reduced triforine sensitivity and four isolates classed as triforine sensitive were selected for cross-resistance studies to three other DMI fungicides, 500 g/kg bitertanol (Baycor), 100 g/litre cyproconazole (Alto) and 200 g/kg flusilazol (Nustar). Preparation of fungicide-amended PDA, sensitivity tests and EC_{50} calculations for each isolate were carried out as above. Final fungicide concentrations in PDA were; 0, 0.1, 0.3, 1, 3, 10 and 30 mg/litre for triforine and 0, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, and 1 mg/litre for bitertanol, cyproconazole and flusilazol. In this test one of the four isolates initially identified as having reduced sensitivity to triforine, reverted and was subsequently included in the group of sensitive isolates for analysis. Analysis of variance was used to compare the mean EC_{50} of the five sensitive isolates to the mean EC_{50} of the three isolates showing reduced sensitivity to triforine.

Pathogenicity and fungicide efficacy tests

Three isolates with significantly reduced triforine sensitivity (EC_{50} values 9.2, 22.9 and 13.1 mg/litre triforine) and two isolates classed as triforine sensitive (EC_{50} values 5.1 and 3.8 mg/litre triforine) were selected for use in these studies. Peach (cv. Fairhaven) shoots with 10 to 20 flower buds were harvested, stored and prepared for isolate inoculation using extended vase-life methods (Elmer 1990). Shoots were induced to flower in the laboratory and any unopened flowers or those at petal-fall were removed. Prior to the application of each isolate, triforine (application rate of 19 ml/100 litres) and cyproconazole (application rate of 1.5 ml/100 litres) were applied to flowers at full bloom to run-off with a hand-held pneumatic sprayer (500 ml) and allowed to dry. The control treatment was sterile distilled water plus 0.05% Tween 80. Isolate inoculum was prepared by flooding 6 to 8 day old cultures on V8 juice agar with sterile distilled water plus 0.05% Tween 80. Spore concentrations adjusted to 1×10^5 spores/ml, were applied to each blossom to run-off using a hand-held airbrush (Badger Corp.). Shoots were then incubated for 6 days at 24°C (± 1°C) in transparent plastic chambers attached to plastic pots and misted to maintain high relative humidity within the chamber. Six days after inoculation blight severity was scored visually on five to 15 flowers per replicate using the following key: 0 = nil discolouration of petal; 1 = slight discolouration of petal; 2 = discolouration <50% of petal, anthers beginning to discolour; 3 = discolouration >50% of petal, anthers visibly wilted; 4 = discolouration of the flower >75%; 5 = discolouration 100%.

The experimental design was a randomised block with 15 treatments per block, replicated four times. Analysis of variance was used to compare differences between treatments.

RESULTS

Orchard survey

In 1992, the triforine sensitivity of *M. fructicola* populations from two of the nine stone fruit orchards tested showed similar mean EC_{50} values and ranges to the triforine sensitive home garden population collected in 1990. Compared to these two sensitive orchards, the other seven showed significantly ($P<0.01$) reduced sensitivity to triforine (results for one sensitive orchard, Orchard 2, and two with reduced sensitivity to triforine, Orchards 8 and 9, are presented in Table 1). The EC_{50} of individual isolates from the home garden population ranged from 0.5 - 10.8 mg/litre triforine, compared to 2.1 - 25.3 mg/litre triforine for isolates from orchards with reduced triforine sensitivity (Table 1). In 1994 the four orchards tested showed similar mean EC_{50} values and ranges to orchards previously found to have reduced triforine sensitivity(results for two of these orchards are presented in Table 1).

TABLE 1: Triforine sensitivity of populations of *Monilinia fructicola* from a home garden population and three Hawkes Bay orchards surveyed in 1992 and two orchards in 1994.

Source	No. of isolates sampled	Mean log EC_{50}	Range
1990 Season			
Home garden	27	0.519 (3.3)[a]	(0.5 - 10.8)[a]
1991/92 season			
Orchard 2	26	0.658 (4.5)	(0.6 - 9.8)
Orchard 8	22	0.860 (7.2)	(3.1 - 25.3)
Orchard 9	31	0.900 (7.9)	(2.4 - 15.6)
LSD (5%)		0.143	
1993/94 season			
Orchard M	30	0.811 (6.5)	(2.7 - 12.4)
Orchard G	29	0.842 (7.0)	(3.4 - 17.3)
LSD (5%)		0.093	

[a] Backtransformed values

Cross-resistance tests

Three isolates with significantly reduced sensitivity to triforine were also significantly ($P<0.01$) less sensitive to three other DMI fungicides tested, when compared to five isolates which were sensitive to triforine (Table 2). Resistance factors for bitertanol, cyproconazole and flusilazol were 2.2, 4.7, and 4.0 respectively.

TABLE 2: Sensitivity of *Monilinia fructicola* isolates to four DMI fungicides. Entries = mean log EC_{50} values, backtransformed values in brackets.

	Mean log EC_{50}			
	triforine	bitertanol	cyproconazole	flusilazol
Five Triforine-sensitive isolates	0.642 (4.4)	-1.132 (0.074)	-2.315 (0.0048)	-1.220 (0.060)
Three isolates with reduced sensitivity to triforine	0.967 (9.3)	-0.796 (0.160)	-1.647 (0.0225)	-0.619 (0.240)
LSD(5%)	0.007	0.133	0.333	0.303
Resistance factor[1]	2.1	2.2	4.7	4.0

[1] Resistance factor = $\frac{EC_{50} \text{ of isolates with reduced triforine sensitivity}}{EC_{50} \text{ of triforine sensitive isolates}}$

Pathogenicity and fungicide efficacy tests

In the absence of fungicide treatments, there was no difference in the ability of isolates with reduced triforine sensitivity and sensitive isolates to cause blossom blight (Table 3). Triforine and cyproconazole both significantly ($P<0.01$) reduced blossom blight severity compared to the water control, when pl each blossoms were challenged with both sensitive isolates and isolates with reduced triforine sensitivity (Table 3). In addition, there was no significant difference between the two fungicides in terms of blossom blight control.

TABLE 3: Mean blight severity of *M. fructicola* on peach blossom treated with either DMI fungicides or water.

Strain[1]	water	triforine	cyproconazole
R1	4.53	0.22	1.03
R2	4.02	0.37	0.35
R3	4.84	0.51	0.50
S1	4.53	0.49	0.25
S2	4.80	1.17	0.36
LSD (5%)	0.89		
Mean	4.45	0.55	0.50
LSD (5%)	0.39		
Significance of Contrasts			
Water vs. Fungicide	**		
triforine vs. Cyproconazole	ns		

[1] Isolates with reduced triforine sensitivity (R1 - R3); triforine sensitive isolates (S1 and S2).

DISCUSSION

Base level sensitivities of *M. fructicola* to triforine were established in 1990 for a population of home garden isolates gathered from throughout New Zealand (Elmer *et al.* 1992). Compared to the home garden population reduced sensitivity to triforine has now been detected during three separate surveys of commercial Hawkes Bay orchards in 1990, 1992 and 1994. The sensitivity of isolates in these orchards was similar, which suggests that there have been no major changes in triforine sensitivity over that time. When isolates with reduced sensitivity were transferred by inoculation through four successive generations on nectarine fruit, the EC_{50} values significantly reduced, which suggested that the isolates were unstable (Braithwaite *et al.* unpublished). This instability could explain why further reductions in sensitivity to triforine have not occurred over the 4 year sampling period.

The magnitude of the shift in sensitivity to triforine was small, with resistance factors ranging from 1.7 to 2.4. Delp and Dekker (1985) suggested that resistance factors of 2.5 or more indicate the emergence of a resistant sub-population. Our results suggest that sub-populations with significantly reduced triforine sensitivity have emerged, but to describe these sub-populations as resistant to triforine is probably not justified. The resistance factors we have detected are on the margin of Delp and Dekker's (1985) criteria and therefore we have used the term "significantly reduced sensitivity". Evidence to date suggests that emergence of resistance to the DMI's occurs in a step-wise manner if DMI selection pressure is maintained over time (Koller and Scheinpflug 1987). Our data suggests that this process may have started to occur in populations of *M. fructicola* in some Hawkes Bay orchards.

We detected cross-resistance to other DMI fungicides used to control brown rot in New Zealand. Cross-resistance within the DMI group has previously been reported for other fungi. Huggenberger *et al.* (1984) detected cross-resistance within the DMI group for *Sphaerotheca fuliginea* on cucurbits. More recently cross-resistance to the DMI's has been detected for *M. fructicola* (Nuninger-Ney 1989), *Pyrenophora teres* (Sheridan and

Nendick 1992), *Venturia inaequalis* (Whelan *et al.* 1992) and *Rhynchosporium secalis* (Kendall *et al.* 1993).

The application of triforine or cyproconazole to peach flowers significantly reduced blight severity for both the sensitive isolates and isolates with reduced sensitivity, suggesting there has been no loss of disease control associated with the reductions in DMI sensitivity. However, disease control on fruit has not been investigated and may need further work.

M. fructicola isolates with reduced triforine sensitivity and sensitive isolates were equally capable of causing blossom blight on untreated flowers, suggesting no loss of fitness in the isolates with reduced sensitivity. Similar results were reported with *R. secalis* on barley inoculated with isolates showing reduced DMI sensitivity (Kendall *et al.* 1993).

Even though the change in sensitivity by *M. fructicola* to the DMI's was small and there appears to be no loss of disease control on blossom, these findings must be interpreted as a warning. There is now a need to reduce DMI selection pressure. High numbers of DMI fungicide applications have been applied to stone fruit orchards in the past and up to 11 applications were reported at one orchard in one season (Dr G. Tate, pers. comm.). This exceeds current recommendations for resistance management of this fungicide group (Prince *et al.* 1989). DMI sensitivity levels in *M. fructicola* populations should continue to be monitored, and resistance management strategies need to be revised.

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