CHANGES IN TRIFORINE SENSITIVITY IN POPULATIONS OF *MONILINIA FRUCTICOLA* FROM HAWKES BAY ORCHARDS

P.A.G. ELMER¹, M. BRAITHWAITE² and D.J. SAVILLE¹

¹MAF Technology, P.O. Box 24, Lincoln, Canterbury,

²Plant Protection Centre, MAF Quality Management, P.O. Box 24, Lincoln, Canterbury.

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Repeated fungicide applications are required in New Zealand orchards to control brown rot of stone fruit caused by *Monilinia fructicola* (Wint.) Honey. Current strategies recommend no more than three demethylation inhibiting (DMI) fungicides over the flowering period and non DMIs at all other times (Prince *et al* 1989). The DMI fungicides registered for brown rot control are bitertanol, fluzilazole, prochloraz and triforine.

The emergence of DMI resistance in some host/pathogen systems (Koller and Scheinpflug 1987) indicates that this group of fungicides may be at risk of resistance development. The isolation of DMI resistant strains of *M. fructicola* in laboratory studies (Gilpatrick 1981; Nuninger Ney *et al* 1989) and the present trend towards DMI applications over the flowering (bitertanol and fluzilazole) and pre-harvest periods (triforine) may suggest an increased risk of selection for DMI resistance in New Zealand orchards (Elmer 1990).

Disease control failures, thought to be associated with repeated triforine applications were reported in several Hastings orchards in 1990 (K.G. Tate pers. comm.). Our objective was to determine whether a change in triforine sensitivity had occurred in a sample of affected orchards.

Fruit infected with *M. fructicola* were sampled randomly from each of three Hastings orchards in 1990, using a stratified sampling pattern (Delp *et al* 1986). A wild population of *M. fructicola* isolates was obtained by sampling infected fruit from unsprayed trees from North and South Island home gardens. Pure cultures of *M. fructicola* were obtained from sporulating fruit by transfer of conidia with a sterile needle to potato dextrose agar (PDA). Actively growing colonies of *M. fructicola* were then transferred to V8 juice agar, sealed and stored at 3°C before bioassay tests.

A solution of the DMI triforine (Saprol, 190 g ai/litre) was prepared in sterile distilled water and diluted with autoclaved 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. 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 sensitivity tests. Two colony diameters were measured at right angles to each other after incubation at 25°C ($\pm 0.5^\circ\text{C}$) in the absence of light for 4 days and the growth rate (mm /day) was calculated.

The sensitivity of each isolate was expressed as an EC₅₀ value calculated from a dose response curve of growth rate (mm/day) to log₁₀ triforine concentration (mg/litre). The mean EC₅₀ of each sample from the orchard populations was compared with the mean EC₅₀ of the population from home gardens using analysis of variance.

The mean EC₅₀ of the population from home gardens was 3.4 mg/litre triforine and the EC₅₀ of individual isolates ranged from 0.5 to 10.8 mg/litre (Table 1). In comparison, the mean EC₅₀ of the orchard populations varied from 4.8 to 8.6 mg/litre and the EC₅₀ of individual isolates ranged from 0.4 to 29.2 mg/litre. The triforine sensitivity of each orchard population was significantly ($P < 0.05$) less than that of the population from home gardens.

The results suggest that a significant ($P < 0.05$) shift in mean sensitivity to the DMI triforine has occurred in *M. fructicola* populations in three New Zealand orchards.

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Although the magnitude of the shift is small (Resistance factor of 2.5, as defined by Delp and Dekker (1985)) evidence from other host/pathogen systems confirms that the emergence of DMI resistant pathogen populations proceeds in a gradual stepwise manner (Koller and Scheinpflug 1987).

TABLE 1: Triforine sensitivity of populations of *M. fructicola* from home gardens and Hastings orchards (1990/91 season).

Source	Number of isolates	Mean Log EC ₅₀ (mg/L)	EC ₅₀ Range	Significance
Home gardens	27	0.534 (3.4) ^a	(0.5 - 10.8) ^a	
Orchard 1	38	0.684 (4.8)	(0.4 - 27.4)	* ^b
Orchard 2	54	0.862 (7.3)	(1.4 - 29.2)	**
Orchard 3	49	0.934 (8.6)	(4.5 - 28.3)	**
LSD (5%) ^c		0.129		
LSD (1%)		0.172		

^a Backtransformed values are presented in brackets.

^b *, ** significantly different from the population from home gardens at P<0.05 and P<0.01.

^c Because of unequal sample sizes an average LSD is presented, based on a mean sample size of 40.

The emergence of triforine resistant strains of *M. fructicola* in the laboratory or field does not necessarily mean that disease control failures will occur. This will only occur if resistant strains dominate the pathogen population. The selection of resistant strains is likely to proceed more rapidly when the pathogenicity, virulence, fitness and competitive ability of resistant strains are similar to sensitive strains. In preliminary *in vivo* studies, the virulence and pathogenicity of *M. fructicola* isolates were investigated in the presence and absence of full field rates of triforine. The resistant isolates were less virulent than a sensitive standard isolate on untreated nectarine (cv Goldmine) fruit segments but they were pathogenic on segments dipped in triforine (Elmer and Braithwaite unpublished).

Our finding of a significant shift in triforine sensitivity in all three orchards sampled has important implications for future brown rot control strategies. There is now a need to reduce selection pressure on *M. fructicola* populations and this may be achieved by strict adherence to current recommendations for the DMI fungicide group (Prince *et al* 1989) as evidence suggests that this has not occurred (Tate and Manktelow pers. comm.). In addition, research is required to investigate the biological characteristics (pathogenicity, fitness, genetic stability and competitive ability) of the triforine resistant isolates of *M. fructicola* and the occurrence of cross-resistance to other DMI fungicides.

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