POPULATION DYNAMICS OF DICARBOXIMIDE RESISTANT STRAINS OF *MONILINIA FRUCTICOLA* AND CONSERVATION OF THE DICARBOXIMIDES FOR BROWN ROT CONTROL

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SUMMARY

The population dynamics of *Monilinia fructicola* strains were investigated in controlled environments and in the field. Frequency of application of dicarboximide fungicides increased the proportion of resistant strains. The incidence of resistant strains at individual sample sites differed between successive growing seasons. There was no correlation between brown rot incidence and the proportion of resistant strains. Without dicarboximide selection pressure the EC₅₀ of some resistant strains decreased *in vivo* after nine generations on fruit. Resistant strains were as virulent and fit as sensitive strains on flowers, but less virulent and fit on fruit. Resistant strains were less competitive than sensitive strains in mixed inoculum tests on flowers and fruit. Results suggest that resistant strains have low persistence at individual sites, but new resistant strains are selected readily.

INTRODUCTION

The occurrence of strains of *Monilinia fructicola* (Wint.) Honey (the causal agent of brown rot of stone fruit) resistant to dicarboximide fungicides has been reported in New Zealand previously (Elmer and Gaunt 1986). However, there was no evidence of disease control failures and dicarboximide resistant strains had not dominated the pathogen population (Elmer and Gaunt 1988). Several biological and management factors may influence the build-up of resistant strains in field populations, including the frequency of fungicide applications, genetic stability, virulence, fitness and the ability to compete with sensitive strains in the absence of fungicide.

This paper reports field and controlled environment studies of the population dynamics of dicarboximide resistant strains of *M. fructicola*. The management of resistant strains in New Zealand orchards is discussed.

MATERIALS AND METHODS

A mass screening system was developed to investigate the population dynamics of resistant strains in New Zealand orchards (Elmer and Gaunt 1988).

Field studies

At one orchard (Blenheim), dicarboximide fungicides were applied in the pre-harvest period in 1987. Five mummified fruit were sampled at random from each of 192 sample sites after harvest from a predetermined pattern. The trial area was then split for dicarboximide treatment for the 1988 growing season and sampled at harvest and post-harvest. In accordance with recommendations for export fruit at that time, the grower applied non-dicarboximides during the flowering period and dicarboximides in the pre-harvest period (modified programme) to one half of the block. A full programme (dicarboximide applications over both the flowering and pre-harvest periods) was applied to the other block. Monitoring of resistant populations continued in 1989, when a modified programme was applied to both the blocks. The frequency of dicarboximide applications was decided by the grower, taking into account weather and disease

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conditions in each year. Dicarboximides were applied with conventional airblast sprayers applying a minimum of 2,000 litres/ha.

Sampling was carried out in five other orchards from Central Otago to the Waikato region to investigate survival and persistence of resistant strains. One of these received a modified and full programme in split blocks (Elmer 1990).

The proportion (%) of dicarboximide resistant strains was determined by growth and sporulation on potato dextrose agar containing 5 mg/litre iprodione (Elmer 1990), in each block in 1987, 1988 and 1989.

Controlled environment studies

The stability of the level of resistance to dicarboximides was determined (Elmer 1990) for two dicarboximide sensitive strains and 11 dicarboximide resistant strains by measuring sensitivity to iprodione (Rovral FLO 250 gm/litre) before (G0) and after (G9) nine successive transfers of inoculum on nectarine fruit (cv. Fantasia). Dicarboximide sensitivity, expressed as EC_{50} values, was calculated at G0 and G9 from dose-response curves, and a t-test was used to determine if a change in EC_{50} had occurred from G0 to G9.

The virulence, fitness and competitive ability of a representative sensitive strain (S1) and two representative strains (R1 and R3) were investigated on host tissues. Spore suspensions (1×10^5 spores/ml), derived from V8 juice agar cultures were applied to opened peach blossoms (cv. Redhaven) to run-off. The number of spores produced per flower was measured at regular intervals after incubation at 21°C ($\pm 1^\circ\text{C}$) with a 14 hour photoperiod of fluorescent and incandescent light. Nectarine fruit (cv. Late Fantasia) of uniform maturity were wounded to a constant depth (5 mm) and diameter (6 mm), before inoculation with $50 \mu\text{l}$ of a spore suspension (2×10^5 spores/ml). Lesion diameter (mean of two measurements at right angles to each other) and spore production were measured at regular intervals after incubation at 25°C ($\pm 0.5^\circ\text{C}$) with a 12 hour photoperiod of fluorescent and near ultra-violet light. Lesion growth rates (mm/hour) and sporulation rates (spore number/flower/hour and spore number/fruit/hour) were derived from linear regression equations. The slope parameters for paired strains were tested for differences using a t-test.

In competition tests spore suspensions of S1, R1 and R3 were prepared separately as described above. Suspensions were mixed to give a proportion of resistant strains ranging from 0% to 100%, then applied to run-off to flowers and fruit and incubated as described above. A bioassay was used to determine the proportion of resistant and sensitive strains in populations sampled from flowers and fruit (Elmer 1990).

Slope parameters derived from regressions of final and initial proportions of resistant strains, were used to describe competitiveness. A t-test was used to test slopes for the null hypothesis of equal competitive ability (slope parameter = 1.0).

RESULTS

Field studies

Data for the Blenheim orchard is presented in Figure 1. The proportion of resistant strains was relatively low at the post-harvest sample in 1987 and there was no significant difference ($P > 0.05$) between the two blocks. At the post-harvest sample in 1988 the proportion of resistant strains was significantly greater ($P < 0.05$) in the block which received a higher frequency of dicarboximide applications (full programme), compared to the block which received fewer dicarboximide applications (modified programme). Conditions were favourable for disease development at the Blenheim orchard in 1989. At the harvest sample the proportion of resistant strains was significantly greater ($P < 0.01$) in the block which received more frequent dicarboximide applications, than the block which received fewer dicarboximide applications in 1988, despite the orchardist applying the same recommended modified programme to both blocks in 1989.

The proportion of resistant strains declined in the absence of dicarboximide selection pressure between the harvest and post-harvest samples in both 1988 and 1989 (Fig. 1). The decline was significant ($P < 0.05$) in the modified block in 1988 and the full block in 1989.

Fig. 1: The proportion of dicarboximide resistant strains of *M. fructicola* in the Blenheim trial orchard.

¹ Full dicarboximide programme (dicarboximides applied at flowering and preharvest).

² Modified dicarboximide programme (non-dicarboximides applied at flowering and dicarboximides applied pre-harvest).

³ Analysis of harvest (H) and postharvest (PH) samples within each year.

⁴ Analysis of the modified and full blocks (*, **, *** Significantly different at $P < 0.05$, $P < 0.01$, $P < 0.0001$. Chi-square value is 3.8, 6.6 and 10.8 respectively).

In the investigation of survival and persistence of resistant strains there was no significant correlation ($P > 0.05$) in incidence of resistant strains between successive seasons at sample sites in both the modified and full blocks.

Controlled environment studies

Dicarboximide resistance was stable over nine generations on fruit for the majority of resistant strains tested, as shown by strain R3 (Table 1). However, the EC_{50} decreased significantly ($P < 0.05$) in four resistant strains, as shown by R1.

Resistant strains were as virulent and fit as sensitive strains on flowers, as determined by rate of spore production (Table 1). The resistant strains were significantly ($P < 0.05$) less virulent and fit than sensitive strains on fruit, as measured by lesion diameter and rate of spore production (Table 1). Resistant strains were significantly ($P < 0.01$) less competitive in mixed inoculations with sensitive strains on flowers and fruit (Table 1).

DISCUSSION

Increased dicarboximide sensitivity in some resistant strains, reduced virulence and fitness on fruit, and poor competitive ability of resistant strains in the absence of dicarboximide were all consistent with the observed decline of the proportion of resistant strains in field populations. The very low competitive ability of resistant strains on fruit (eg R3) suggests that the decline of resistant strains in a pathogen population may be rapid, even when a substantial proportion of the population is resistant before competition. Despite low competitiveness on fruit, resistant strains did not disappear from the population in laboratory experiments. This agrees with field observations that resistant strains did not disappear from field populations even though their proportion declines in the absence of dicarboximide selection pressure.

A reduction in selection pressure by reduced frequency of dicarboximide applications may delay or avoid the build-up of resistant strains in populations of *M. fructicola*. Current recommendations for dicarboximide resistance management and brown rot control advise not more than three pre-harvest applications of dicarboximide, and the use of non-dicarboximides during the flowering period to shuck-fall (Elliott *et al* 1988).

TABLE 1: The stability of dicarboximide resistance, virulence, fitness and competitive ability of strains of *M. fructicola*.

Strain ¹	EC ₅₀ (mg ai/litre iprodione)			Blossom blight	Fruit rot			Competition	
	Initial ²	Final ³	t-test ⁴		Rate of spore production/flower/hour (x10 ⁻³)	Rate of lesion growth (mm/hour)	Rate of spore production/fruit/hour (x10 ⁻⁶)	Flowers	Fruit
S1	0.3	0.1	*	16 ⁵	0.9 ⁵	1.4 ⁵			
R1	79	18	**	10 ns ⁶	0.5 ***	0.2 ***	0.67***	0.47***	
R3	159	161	ns	16 ns	0.3 **	0.003**	0.7 **	0.2 **	

¹ Sensitive (S, PDDCC # 7640) and representative resistant (R) strains.

² Sensitivity before fruit inoculation.

³ Sensitivity after nine successive transfers of conidia on nectarine fruit (cv. Fantasia).

⁴ Analysis of difference between initial and final EC₅₀ values.

⁵ Derived from the regression equation, $Y = a + b$ (hours).

⁶ Analysis of difference between S1 and resistant strains by a t-test.

⁷ Slope (b) derived from the regression equation, (final proportion of resistance = $a + b$ (initial proportion of resistance)) in mixed inoculum tests with S1.

⁸ Slope (b) significantly different from $b = 1.0$ (slope of equal competitive ability) using a t-test.

*, ** significantly different at $P < 0.05$ and $P < 0.01$

ns = not significantly different.

A more integrated approach to disease and resistance management is required in New Zealand orchards, to extend the effective life of dicarboximides and maintain disease control, particularly in seasons and locations conducive to disease development. Two key concepts form the basis of future strategies. First, the larger the pathogen population the greater the risks of resistance. Second, more frequent applications of fungicide increase the risk of resistance, especially if these are used throughout the season. A reduction in the size of the pathogen population requires greater emphasis on good orchard practices. The hedgerow system associated with minimal pruning may provide a favourable environment for disease development and requires reassessment. Cultivars prone to splitting and late maturing cultivars are likely to increase the pathogen population in an orchard. The integration of partial host resistance and reduced frequency of fungicide applications has been advocated for cereals (Wolfe *et al* 1988). Several stone fruit cultivars were reported to be partially resistant to the *Monilinia* spp. (Byrde and Willets 1977). Recently, two partially resistant cultivars of peach were reported (Feliciano *et al* 1987; Adaskaveg *et al* 1989).

Options for reducing the frequency of dicarboximide applications in the pre-harvest period include the use of mixtures or alternation of dicarboximide and non-dicarboximide fungicides. The choice of dicarboximide mixtures and appropriate non-dicarboximide fungicides may be severely restricted because of cost and the lack of suitable companion compounds with export residue tolerances. Ogawa (1983) expressed doubts on the efficacy of mixtures to delay resistance in *M. fructicola* populations. Mixtures of dicarboximide and benzimidazole have been applied in other host pathogen systems, but there is a risk of selection of dual resistant strains. Strains of *M. fructicola* resistant to both benzimidazole and dicarboximide were obtained with relative ease in laboratory studies (Elmer and Gaunt 1986). Alternation or a mixture of dicarboximide fungicides with triforine is practically possible in North Island orchards where benzimidazole resistant strains have been isolated. In South Island orchards the use of dicarboximides alternated with benzimidazole/triforine applications warrants further investigation. However, increased frequency of benzimidazole applications in some

South Island orchards indicates a need to monitor *M. fructicola* populations for benzimidazole resistance. The present trend towards several demethylation inhibitor (DMI) applications over the flowering period and triforine (a DMI) pre-harvest, may select for DMI resistant strains in New Zealand orchards. A report of DMI resistant strains of *M. fructicola* selected in the laboratory (Nuninger-Ney *et al* 1989), and more frequent triforine applications indicates a need to extend resistance monitoring to the DMI's.

Field studies supported laboratory findings that dicarboximide resistant strains have not yet acquired all the necessary characteristics to dominate field populations. However, virulence and fitness are dynamic characteristics which should not be regarded as static (Wolfe 1982). Continued dicarboximide applications may select resistant strains with equal virulence and fitness. Future monitoring programmes should therefore be based on monitoring both the frequency and the fitness of dicarboximide resistant isolates.

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