DICARBOXIMIDE RESISTANCE AND DISEASE CONTROL IN BROWN ROT OF STONEFRUIT

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SUMMARY

The EC₅₀ values of the majority of field isolates resistant to dicarboximides ranged from 10-90 mg/litre iprodione. The fitness of resistant strains on blossom and fruit was similar to that of sensitive strains. The frequency of application of dicarboximide fungicides during the growth season influenced the incidence of resistant strains. However, there was little correlation between resistance and brown rot incidence. These results suggest that dicarboximide fungicides, associated with other control methods and monitoring of resistance, can continue to be used with caution.

INTRODUCTION

Strains of *Monilinia fructicola* (causal agent of brown rot of stonefruit) resistant to dicarboximide fungicides were detected in an Australian orchard (Penrose *et al* 1985) and New Zealand orchards in 1985 (Elmer and Gaunt 1986). Spray programmes were changed in New Zealand in 1986, with reduced use of dicarboximides, in an attempt to prolong their effective life and reduce the selection pressure for resistant strains. The emergence of resistant strains does not necessarily cause disease control failures. These occur only if resistant strains are genetically stable, pathogenic, able to overwinter and compete with sensitive strains during the crop season. The selection of resistance in the field is dependant upon the biological characteristics of resistant strains and several management factors (Koller and Scheinpflug 1987).

This paper reports studies of the degree of resistance to dicarboximides and the pathogenicity and fitness of dicarboximide resistant strains of *M. fructicola*. The effectiveness of reducing the frequency of dicarboximide applications was monitored in several orchards and the correlation between resistance incidence and brown rot was investigated.

MATERIALS AND METHODS

Level of resistance, pathogenicity and fitness

The sensitivity of 14 sensitive strains, isolated from unsprayed trees in home gardens in Christchurch, and 19 resistant strains from stonefruit production areas in New Zealand was measured.

In sensitivity tests 6 mm diameter plugs from the margin of actively growing 6-9 day old potato dextrose agar (PDA) cultures incubated in the dark were placed mycelial surface down on test media containing different concentrations of iprodione (Rovral FLO). Sensitivity was expressed as EC₅₀ and EC₉₅ values calculated from dose response curves.

One sensitive and two resistant strains were selected for further analysis of biological characteristics measured on PDA and host tissues. All cultures were incubated at 25 °C (±0.5 °C). Spore production on PDA was measured when colony growth reached the edge of inoculated plates. Spore germination on PDA was measured after 4 hours incubation.

Spore suspensions were prepared for host tissue inoculations from cultures grown on V8 juice agar. Suspensions (1x10⁷ spores/ml) were applied to opened peach blossoms (cv. Redhaven) to run-off and the incubation period, latent period and spore numbers per flower were measured after incubation at 21 °C (±1 °C). Nectarine fruit (cv. Late

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Fantasia) of uniform maturity were wounded to a constant depth and diameter (5 mm) prior to inoculation with spore suspension. Incubation period, latent period, lesion area and spore production were measured after incubation at 25 °C (± 0.5 °C).

Mass screening system and surveys

A system was developed to test more than 1,000 isolates per orchard. Iprodione (250 g/litre) and streptomycin sulphate were added to autoclaved PDA at 50-55 °C and dispensed (5 ml) in sterile plastic lipstick cartridges. The final concentration of iprodione was selected to detect resistant strains by visible growth, based on EC₂₅, EC₅₀ and EC₉₅ data. The system was verified initially in laboratory tests with resistant and sensitive strains. Twenty resistant isolates detected from one orchard using the cartridge system were tested further on 0 and 5 mg/litre iprodione for verification of field tests. Spores removed from 15 fruit selected at random from each orchard were plated onto unamended PDA to confirm spore viability in 1987 and 1988.

In 1987 five to ten mummified fruit were sampled at random after harvest from a predetermined pattern and size of sample unit (one or two trees) from six orchards. The same sites were revisited and sampled in the same way after harvest in 1988. At sample units where resistance was recorded in 1987 (maximum of 20) healthy fruit (20-32) were picked at random. This was repeated for an equal number of 'sensitive' sites. Harvested fruit were incubated at 25 °C ± 1 °C, assessed for disease every 24 hours for 6 days and any brown rot lesions were tested for resistance.

Sampled fruit were tested directly by gently touching a freshly sporulating area with the extruded end of the agar core in a cartridge. Agar disks (12 mm x 2 mm) were aseptically removed and placed in sterile plastic petri dishes. When no fresh conidia were visible, fruits were surface sterilized (0.5% v/v sodium hypochlorite plus 0.05% v/v Tween 20), soaked in a sterile sucrose solution (10% w/v) for 8 hours and incubated at 23 °C ± 2 °C for 72 hours. The freshly produced conidia were sampled as before. Resistance was confirmed visually by the presence of monilinia-type conidia up to 4 days after incubation.

RESULTS

The EC₅₀ based on mycelial growth on agar media, ranged from 0.3 to 0.7 mg/litre iprodione for sensitive strains and from 3 to 90 mg/litre for resistant strains. One resistant strain (R2) was similar to a representative sensitive strain (S1) for all variables measured except growth rate on PDA (Table 1). The other resistant strain (R1) was similar to S1 for two variables on PDA and one variable on blossoms and fruit, but was significantly ($P = 0.05$) less fit on host tissues.

TABLE 1: A comparison of the fitness and pathogenicity of dicarboximide sensitive and resistant strains of *M. fructicola*.

Strain ¹	Potato Dextrose Agar			Blossom Blight		Fruit rot		
	Spore germination [%]	Growth rate (mm/hour)	Spore number ($\times 10^{-3}$ /mm ²)	Latent period (hours)	Spore number $\times 10^{-6}$ /flower)	Latent period (hours)	Lesion area (mm ² $\times 10^{-3}$)	Spore number ($\times 10^{-6}$ /mm ²)
S1	99	0.35	0.3	40	0.5	37	2.3	0.04
R1 (77)	97	0.68	0.2	40	0.2	42	1.3	0.01
R2 (156)	92	0.69	—	36	0.7	38	2.8	0.05
LSD($P=0.05$)	8.2		0.15		0.24	25.3	3.42	0.018

¹ Sensitive (S,PDDCC #7640) and resistant (R) strains. Figures in parenthesis are resistance factors calculated from EC₅₀ data relative to S1.

Six orchards were monitored and data for four representative blocks are presented in Table 2. The proportion of resistant strains was low in 1987 (Table 2). Increased frequency of dicarboximide applications significantly ($P = 0.05$) increased the

proportion of resistant strains and the proportion of sample units with resistant strains. At two orchards the proportion of resistant strains declined between harvest and postharvest samples in 1988. The proportion of resistant strains increased slightly between 1987 and 1988 with the current recommended programme in which dicarboximides were applied pre-harvest only. Brown rot incidence at harvest was significantly greater at resistant sites than at sensitive sites in two orchards. However there was no correlation ($P=0.05$, $r=0.18-0.31$) between brown rot incidence and the proportion of resistant strains at resistant sites.

TABLE 2: The proportion of resistant strains in *M. fructicola* populations and disease incidence on fruit in stonefruit orchards in New Zealand.

Site	Dicarboximide ¹ programme	Total number of isolates		% Resistant strains		% of sample units with resistant strains				Mean % brown rot at harvest 1988 ⁴		
		'87	'88	'87	'88	'87	'88	'87	'88	R	S	
		PH ²	H ³	PH	PH	H	PH	PH	H	PH	Sites	Sites
Blenheim	Modified	495	298	294	4	10	5	18	40	16	44	30
	Full	499	315	328	5	15	11	20	70	26	43	37
Christchurch	Modified	529	269	288	4	7	6	14	12	16	38	27
	Full	979	227	680	0.2	11	16	2	23	58	5	7

¹ "Modified" (dicarboximides applied pre-harvest only) "Full" (dicarboximides applied at flowering and pre-harvest).

² Post-harvest assessments sampled 4 to 6 weeks after harvest (1987 and 1988).

³ Harvest assessments tested 2 to 6 days after harvest and incubation.

⁴ Fruit harvested on 7/2/88, 19/2/88 and 7/4/88 for Blenheim, Christchurch and Clyde respectively.

R Resistant

S Sensitive

DISCUSSION

The characteristics of dicarboximide resistance in *M. fructicola* are different to the characteristics of benzimidazole resistance in *M. fructicola* and dicarboximide resistance in *Botrytis cinerea*. Disease control failures associated with benzimidazole resistance in *M. fructicola* were the result of selection of strains with high levels of resistance (resistance factor [RF] >1,000). Strains were genetically stable, pathogenic, fit and able to compete successfully in mixed inoculations (Penrose *et al* 1979). Recently, benzimidazole resistant strains were isolated from Hastings orchards 9 years after the last use of benzimidazole sprays (N. Sanoamuang pers. comm.). Dicarboximide resistance in *M. fructicola* differs in that levels of resistance are lower (RF = 6-200), pathogenicity and fitness varies, and a proportion of strains are unable to colonize fruit. The inability to rot fruit may be related to abnormal sensitivity to high osmotic pressures commonly associated with ripening fruit (Beever 1983). A preliminary investigation has found abnormal sensitivity to high osmotica in one strain. Initial studies have shown also that some dicarboximide resistant strains were less competitive in mixed populations with a sensitive strain, even though, individually, they were apparently fit and pathogenic. Application of full field rates of iprodione prior to pathogen inoculation failed to protect flowers against blossom blight by resistant strains, but spore production was significantly ($P = 0.05$) reduced.

Dicarboximide resistance in *B. cinerea* is different to that in *M. fructicola*. *B. cinerea* is a multi-host pathogen with many generations per season, whereas *M. fructicola* is a single-host pathogen with fewer generations. The proportion of *B. cinerea* resistant strains in some vineyards is high (100%), low level resistance (RF = 10) is relatively common and resistant strains are generally less fit than sensitive strains (Beever and Bryde 1982; Beever and Brien 1983). Data presented in this paper indicate that some resistant strains of *M. fructicola* were as fit as sensitive strains in laboratory tests. Theoretically these strains could dominate the pathogen population, with disease

control failures resulting, although field evidence suggests that they have not. The level of resistance may not be high enough and full field rates of dicarboximides may affect the epidemiology of resistant strains. Alternatively, resistant strains may be less fit and there is evidence to support this from competition studies. At two orchards using modified programmes (Table 2) selection for resistance appears to be the dominant process that has occurred in the 1988 season rather than a cumulative increase in resistant populations. This has implications for blocks with sequentially maturing cultivars. There is some field evidence to suggest that late maturing cultivars may be at risk from strains selected in earlier maturing blocks.

Monitoring must continue at some sites to detect any further shifts in dicarboximide resistance. In addition there is a need to evaluate preharvest spray programmes with non-dicarboximide fungicides to reduce further the dependency on dicarboximide fungicides.

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