INHERITANCE OF RESISTANCE TO MBC FUNGICIDES IN ISOLATES OF *MONILINIA FRUCTICOLA*

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SUMMARY

Genetic analysis of MBC resistant isolates of *Monilinia fructicola* (Wint.) Honey was carried out on ascospores derived from apothecia produced in the laboratory. Ascospore populations and ascospore sets in linear arrangement indicated that resistance is conferred by a mutation in a single gene, and is affected by modifying genes and gene conversion mechanisms during crossing over. Crossing over was frequent, suggesting that recombination of resistance with other characters such as pathogenicity and fitness, may occur readily. The segregation ratio (1:1) from resistant parents revealed that heterokaryons containing both resistant and sensitive alleles are common in resistant populations and resistance is dominant.

Keywords: *Monilinia fructicola,* inheritance of resistance, heterokaryosis, fungicide resistance, MBC fungicides

INTRODUCTION

The appearance of fungal strains resistant to MBC fungicides has caused much concern (Hartill *et al* 1975). Strains of *Monilinia fructicola* (Wint.) Honey resistant to MBC were first found in North Island orchards in 1979 (Anon. 1980) and the resistance has persisted in the properties, although the products have not been used extensively since 1979 (Sanoamuang and Gaunt 1991).

The genetics of inheritance of MBC resistance has been studied in many fungi (Grindle 1987). However, no information is available on the causal agent of brown rot, perhaps because it is difficult to produce ascospores for analysis in controlled conditions.

Ten isolates of *M. fructicola* from different locations in New Zealand, and resistant to MBC fungicides, were studied. Techniques were developed to form apothecia in the laboratory. The progeny derived from individual isolates were analyzed to determine the number of genes governing resistance, and mechanisms of inheritance of resistance.

MATERIALS AND METHODS

Isolates of *M. fructicola* resistant and sensitive to MBC fungicides, isolated from North Island properties and from nectarines imported from California, USA, were selected. Inoculum of individual isolates, and mixed inoculum (50:50) of selected sensitive and resistant isolates, were inoculated into nectarine (*cv* Fantasia) and peach (*cv* Black Boy). Fruit were surface sterilized, in 0.5% (v/v) sodium hypochlorite plus 0.05% (v/v) Tween 20 for 2.5 minutes, before inoculation. Inoculated fruit were incubated to produce apothecia and ascospores (Sanoamuang and Gaunt unpublished).

Ascospores isolated from macerated apothecial tissue were diluted to 5 x 10^2 ascospores/ml. Aliquots (0.5 ml) of the suspension were plated on a selective medium (potato dextrose agar (PDA) + 100 units/ml of streptomycin sulfate + 0.015 mg/litre of flusilazole) unamended or amended with 0.5 or 1 mg/litre of carbendazim. The ratio of colonies growing on the amended and unamended selective media indicated the proportion of ascospores carrying the resistant character.

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Single ascospores isolated in linear sets from asci (Sanoamuang and Gaunt unpublished) were analyzed for resistance expression. Five hundred and eighty four ascospores from 73 asci were isolated and tested on PDA amended with 1 and 100 mg/litre carbendazim. The number and arrangement of resistant and sensitive ascospores in each ascus indicated the method of inheritance of resistance and the frequency of recombination during meiosis.

RESULTS

All of the ascospores derived from sensitive parents were sensitive to MBC fungicides (Table 1). Three high-resistant parental isolates produced resistant and sensitive progeny in ratios which were not significantly different to a 1:1 ratio ($P<0.05$). A fourth resistant parent produced a similar progeny ratio which was marginally different ($P<0.05$) to a 1:1 ratio. In contrast, low-resistant parent isolates produced progeny which were markedly different to a 1:1 segregation ratio for resistance:sensitivity.

TABLE 1: Frequency of MBC resistant progeny in ascospore populations derived from high resistant (HR), low resistant (LR) and sensitive (S) parental isolates.

Parent isolates	No. of apothecia tested	No. of colonies		Chi-Square Test of ratios		
		R	S	1:1 ($P<0.05$ = 12.7)	9:7	7:9 ($P<0.01$ = 63.6)
S1	4	0	759	—	—	—
S2	4	0	830	—	—	—
S3	3	0	538	—	—	—
S4	3	0	753	—	—	—
HR1	15	1939	2174	13.4*	138.5**	19.2*
HR2	2	230	309	11.5ns	40.1*	0.25ns
HR3	9	1384	1271	4.8ns	18.3*	75.6**
HR4	16	1897	1838	0.93ns	45.1*	75.1**
LR1	6	707	290	174.4**	87.1**	298.8**
LR2	6	597	362	57.5*	13.9*	133.3**

Four crosses between sensitive and high-resistant isolates were attempted. Isolate S1 with isolate HR1 and HR2 produced only sensitive progeny, whereas with HR3 and LR1 both sensitive and resistant progeny were produced, in ratios not different ($P>0.05$) from a 1:1 ratio.

The segregation ratio of ascospores in linear arrangement from individual asci was four resistant: four sensitive phenotypes in most cases (Table 2). Six of 64 asci derived from single parents segregated in the ratio 5:3 and one ascus segregated 6:2. The ascospores derived from attempted crosses (S1 x HR1 and S1 x LR1) produced only sensitive phenotypes.

TABLE 2: Frequency of phenotypic expression, segregation types and segregation ratio (R:S) of ascospore sets in linear arrangement derived from apothecia.

Isolates	Segregation types[1]			Segregation ratios (R:S)			
	PD	NPD	TT	0:8	4:4	5:3	6:2
HR1	0	3	1	0	4	0	0
HR3	7	5	2	0	12	2	0
HR4	9	12	5	0	22	4	0
D1	7	10	3	0	19	0	1
Total	23	30	11	0	57	6	1

[1]PD = parental ditype, NPD = non-parental ditype and TT = tetra type (Fincham *et al* 1979)

The patterns of ascospore arrangement in most of the asci derived from single parents segregated into six patterns. The frequency of the segregation types (parental ditype: non-parental ditype and tetra type) were 23:30:11, respectively.

DISCUSSION

Segregation into resistant and sensitive phenotypes from resistant parents indicates that the mycelium of these isolates was heterokaryotic. The majority of segregation, 1:1, from high-resistant parents and the six patterns of segregation in asci suggested that high resistance of MBC fungicide in *M. fructicola* is governed by a single locus. The deviations from 9:7 or 7:9 ratios (Table 1) is evidence to reject a two locus hypothesis for control of MBC resistance. The resistant gene is dominant because in the heterokaryotic state the only phenotype expressed was resistance.

The ratios of segregation types, 23:30:11, suggested that the position of the locus on the chromosome allowed crossing over to occur in at least 50% of meiotic divisions (Fincham *et al* 1979).

Segregation ratios of 5:3 and 6:2 were observed in seven asci, deviating from the 4:4 ratio. This indicated that conversion occurred during crossing over, and that the conversion was only to the resistant phenotype and not vice versa.

Ascospores derived from attempted crosses produced only sensitive phenotypes, or 1:1 ratio of resistance and sensitive. This suggests that only one parent had produced the sexual progeny. Further investigation of the sexual mechanism is needed to establish whether *M. fructicola* is potentially self-fertile.

There was also evidence (results not presented) of increased resistance to low levels of the MBC fungicide (below 10 mg/litre) in sensitive ascospores derived from sensitive or resistant parents. It is possible, therefore, that modifying genes existed and that they are involved in the resistance mechanism. Quantitative expression of these genes, involved at low fungicide levels, would be masked at high fungicide levels.

These findings suggest that sexual reproduction in *M. fructicola* is important because of the potential for selection of resistance in *M. fructicola* populations and recombination with fitness.

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