SURVEY OF FUNGICIDE RESISTANCE IN *MONILINIA FRUCTICOLA* IN NEW ZEALAND

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SUMMARY

Isolates of the brown rot fungus, *Monilinia fructicola* (Wint.) Honey were obtained from 45 commercial summerfruit orchards and 27 home gardens throughout New Zealand during the 1989/90 growing season. Isolates were screened on fungicide amended agar and benzimidazole resistance was detected from both North and South Island commercial orchards and home gardens. This is the first report of benzimidazole resistance in the South Island. Frequency of benzimidazole resistance varied between individual orchards. Dicarboximide resistance occurred at low frequencies. A dual benzimidazole/dicarboximide resistant isolate was detected from Nelson. Resistance to the demethylation inhibiting fungicides, triforine and bitertanol, was not detected.

Keywords: survey, *Monilinia fructicola*, resistance, benzimidazoles, dicarboximides

INTRODUCTION

Each year the possible presence of exotic pests and the geographic distribution of established pests is monitored on a selected crop of economic importance within New Zealand by MAF Quality Management. A component of these crop surveys is the monitoring of pathogen populations for fungicide resistance, to determine changes in fungicide sensitivity and the geographic distribution of resistant populations.

Adequate control of brown rot of summerfruit, caused by *Monilinia fructicola* (Wint.) Honey was achieved with the introduction of the benzimidazole fungicides in the early 1970's. Following the emergence of benzimidazole resistant strains in North Island summerfruit orchards in 1979 (Anon. 1980), dicarboximide fungicides replaced the benzimidazoles for brown rot control in many New Zealand spray programmes. In 1985, a survey of New Zealand summerfruit orchards confirmed that benzimidazole resistance was still present in the North Island, but not in South Island orchards (Elmer and Gaunt 1986). Recently, benzimidazole resistant strains of *M. fructicola* were detected in Christchurch from nectarines imported from California (Sanoamuang and Elmer, unpublished). The freedom of movement of summerfruit material from areas known to contain benzimidazole resistance, and reports that the frequency of benzimidazole applications have exceeded current resistance management recommendations on some properties, may contribute to the spread and further development of resistant *M. fructicola* populations throughout New Zealand.

Dicarboximide resistant strains of *M. fructicola* were detected in a survey of New Zealand summerfruit orchards in 1985 (Elmer and Gaunt 1986). As a consequence brown rot spray programmes were modified to reduce selection pressure and maintain disease control. In contrast to the situation with the benzimidazoles, research indicated that dicarboximide resistance was a manageable problem (Elmer and Gaunt 1988). However, continued use of dicarboximide fungicides may lead to the selection of resistant strains with the ability to dominate the pathogen population. Therefore, there is a need to continue monitoring *M. fructicola* populations for dicarboximide resistance.

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The demethylation inhibiting (DMI) fungicides (bitertanol, flusilazol and prochloraz) are recommended for control of blossom blight caused by *M. fructicola*. Triforine (a DMI), has also been recommended for season-long brown rot control since 1979. The emergence of DMI resistant strains in other host/pathogen systems (Fiaccadori *et al* 1987) and the isolation of DMI resistant strains of *M. fructicola* in the laboratory (Nuninger-Ney *et al* 1989) indicates that the effective use of DMIs in the future may be a risk if they are used intensively.

The objective of this study was to survey populations of *M. fructicola* on summerfruit from commercial orchards and home gardens for resistance to benzimidazole, dicarboximide and DMI fungicides.

MATERIALS AND METHODS

Commercial orchards and home gardens were sampled in summerfruit producing areas throughout New Zealand including Auckland, Waikato, Hawke's Bay, Nelson, Blenheim, Canterbury, North and Central Otago and Dunedin during the 1989/90 growing season. Commercial orchards were sampled randomly and trees in home gardens were selected at sample sites containing fruit fly traps (Cowley and Frampton 1989). Up to ten brown rot infected shoots or fruits were collected from each sample site and forwarded to the Lincoln Plant Protection Centre laboratory for isolation. Infected shoots were cut into 20 mm lengths, surface sterilized in 0.5% sodium hypochlorite solution and plated onto 1.5% water agar (Davis). Conidia were transferred from sporulating fruit with a sterile needle and plated directly onto potato dextrose agar (PDA). Actively growing colonies of *M. fructicola* were transferred to V8 juice agar plates, sealed and stored at 3 °C prior to bioassay tests.

Fungicide solutions of the benzimidazole, carbendazim (Delsene 75DF, 750g ai/litre); the dicarboximide, iprodione (Rovral FLO, 250 g ai/litre); and the DMIs bitertanol (Baycor 50WP, 500 g ai/litre) and triforine (Saprol, 190 g ai/litre), were prepared in sterile distilled water and added to autoclaved PDA after cooling to 50-55 °C. The amended agar was then dispensed (20 ml) into plastic petri dishes (85 mm diameter) and stored at 3 °C prior to use.

A benzimidazole resistant strain (PDDCC #7640) and dicarboximide sensitive strains (including PDDCC #7642) of *M. fructicola* were used as reference standards in each of eight separate sensitivity tests. Subculturing was by mass transfer of conidia and mycelium. Mycelial plugs (6 mm diameter), from the margin of actively growing 6-10 day old cultures, were placed mycelial surface down on test media and incubated at 25 °C (± 0.5 °C) for 4 days in the absence of light. Colony diameters were measured twice at right angles to each other.

Isolates with no obvious reduction in growth on 1 and 10 mg/litre carbendazim were classed as benzimidazole resistant. Isolates were classed as dicarboximide resistant when the level of growth reduction on 5 mg/litre iprodione was less than or equal to 75% of the growth on non-amended PDA. Isolates with any visible growth on 1 mg/litre bitertanol and 50 mg/litre triforine were classed as DMI resistant.

RESULTS

Results are summarised in Table 1. Eighteen of the 321 isolates (6%) from commercial orchards grew on 1 mg/litre and 10 mg/litre carbendazim. The majority of benzimidazole resistant isolates were detected in the North Island from Auckland, Hawke's Bay and Waikato orchards. In addition, the first occurrence of benzimidazole resistance was detected in the South Island from orchards in Nelson, Central Otago and Dunedin. The frequency of benzimidazole resistance detected at one Dunedin orchard was notably high (73%).

Dicarboximide resistance was detected in commercial orchards from Auckland, Hawke's Bay, Nelson, Canterbury and Central Otago. The frequency of dicarboximide resistance from the Auckland region (29%) was greater than for the other summerfruit growing regions sampled. Dicarboximide resistance was not detected in isolates sampled from Waikato, North Otago or Dunedin. This may reflect the frequency of dicarboximides applied, or the low number of orchards sampled from these regions.

TABLE 1: Fungicide resistance in isolates of *M. fructicola* in New Zealand (1989/90 season).

District	Number of properties assessed	Number of isolates	Percent frequency of resistance in populations for each district	
			carbendazim	iprodione
Commercial Orchards				
Auckland	15	49	22	29
Hawkes Bay	3	34	15	3
Waikato	3	14	21	0
Nelson	2	16	6	6
Canterbury	4	47	0	2
Central Otago	16	141	5	3
North Otago	1	5	0	0
Dunedin	1	15	73	0
Home Gardens				
Auckland	8	47	11	0
Waikato	1	6	0	16
Nelson	1	3	0	0
Canterbury	7	44	0	5
Central Otago	8	47	11	0
North Otago	1	4	0	0
Dunedin	1	7	0	0

An isolate with dual resistance to both the benzimidazole and dicarboximide fungicides was identified from an orchard in Nelson. Resistance to the two DMI fungicides tested (bitertanol and triforine) was not detected in any of the sampled orchards.

One hundred and fifty eight isolates were obtained from 27 home garden properties in Auckland, Waikato, Nelson, Canterbury, Central Otago, North Otago and Dunedin. Benzimidazole resistance was detected at home garden sites in isolates from Auckland (11%) and Central Otago (11%). Dicarboximide resistance was detected in the Waikato (16%) and Canterbury (5%). DMI resistance was not detected at any of the home garden sites sampled.

DISCUSSION

Benzimidazole resistant strains were detected in populations of *M. fructicola* in the South Island of New Zealand for the first time. Investigations showed that the very high frequency of resistant isolates occurring at the Dunedin site had resulted in inadequate brown rot control following benzimidazole applications. The block has subsequently been pulled out. Emergence of benzimidazole resistance in the South Island is likely to have occurred as a result of an increased frequency of benzimidazole fungicide applications. The movement of infected fruit from areas where benzimidazole resistance has been detected may also have contributed. North Island summerfruit orchards or fruit imported from overseas are possible sources of such isolates. Benzimidazole resistance has been detected on imported fruit from California infected with brown rot. However, these isolates were predominantly low level resistant and therefore likely to be genetically different from the benzimidazole resistant populations in New Zealand (Sanoamuang and Elmer, unpublished).

Frequency of benzimidazole resistance in populations of *M. fructicola* in North Island orchards was high, despite a low frequency of benzimidazole applications during the last 10 years. Persistence of benzimidazole resistance in New Zealand orchards is similar to that reported by Penrose (1990) where benomyl-resistant isolates of *M. fructicola* were detected 12 years after their first observation in an orchard in South Australia following low numbers of benzimidazole applications. These results also support laboratory findings that benzimidazole resistant isolates of *M. fructicola* are

equally competitive with the sensitive portion of the population (Sanoamuang and Gaunt 1991). Therefore these benzimidazole resistant isolates are likely to persist within brown rot populations in the absence of benzimidazole selection pressure.

Benzimidazole fungicides are currently recommended for control of brown rot throughout New Zealand. Evidence that the frequency of benzimidazole resistance varied between individual orchards and that the geographic range of benzimidazole resistance now extends to the South Island, indicates that greater caution is required with the use of this group of fungicides to avoid potential disease control failures.

Our finding of a high frequency of dicarboximide resistance in the Auckland region compared with other regions is cause for concern. Dicarboximides (vinclozolin, iprodione and procymidone) failed to protect peach flowers and nectarine fruit from infection caused by dicarboximide resistant isolates *M. fructicola* (Elmer 1990). A more extensive survey of Auckland summerfruit orchards is recommended to determine the relationship between dicarboximide resistance and disease control.

Benzimidazole resistance combined with dicarboximide resistance was reported in laboratory studies (Elmer and Gaunt 1986). The detection of a dual (benzimidazole/dicarboximide) resistant isolate from the field is a significant finding but further research is required to determine the virulence and fitness of this isolate.

DMI resistance was not detected in this survey, but at several Hastings orchards disease control was inadequate following repeated triforine applications during the 1989/90 season. The DMI fungicides tested in this survey were screened at a single concentration which was unlikely to detect small shifts in sensitivity within the pathogen population. Although small shifts in DMI fungicide sensitivity are unlikely to result in control failures they may be an early warning of future resistance development. More intensive screening of isolates over a range of DMI fungicide concentrations is necessary.

The detection of benzimidazole resistance in home gardens suggests that these sites may act as possible sources of inoculum for neighbouring commercial orchards. Therefore surveys of this nature should not be restricted to commercial orchards. The persistence of benzimidazole resistance in the North Island, the detection of benzimidazole resistance in the South Island and the continued presence of dicarboximide resistance in several summerfruit growing regions of New Zealand, strongly supports the need for orchardists to test their summerfruit blocks for fungicide resistance. Significant changes in resistance patterns were detected as a result of this survey, emphasising the importance of the national survey programme and the need for continued monitoring for fungicide resistance on crops throughout New Zealand.

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