A SURVEY OF FUNGICIDE INSENSITIVITY IN *MONILINIA FRUCTICOLA*

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SUMMARY

Isolates of the causal agent of brown rot (*Monilinia fructicola* (Wint.) Honey) were obtained from North and South Island stonefruit orchards. Nineteen percent of the 1292 isolates were dicarboximide insensitive. The frequency of insensitivity in some orchards was greater than that expected from studies of a sensitive strain. Some insensitive isolates grew well on unamended media, sporulated profusely and showed no growth reduction on 1000 mg ai/litre vinclozolin. One of these isolates was pathogenic to peach flowers and nectarine fruit despite application of 500 mg ai/litre vinclozolin before inoculation. Benzimidazole insensitive strains were present only in Hawkes Bay orchards. Fungicide insensitivity is discussed in relation to disease management.

INTRODUCTION

Brown rot, caused by *Monilinia fructicola* (Wint.) Honey, is potentially the most destructive disease of stonefruit (Byrde and Willets 1977). This disease was effectively controlled with benzimidazole fungicides introduced to New Zealand in the early 1970's, but repeated applications of fungicide were required. After predictions that benzimidazole insensitivity would develop in New Zealand (Hartill *et al* 1975), insensitive strains were detected in Australia (Koffman and Penrose 1976) and in North Island orchards in 1979 (Anon. 1980). As a consequence, dicarboximide fungicides replaced benzimidazoles in many New Zealand spray programmes.

Kable (1983) recognised that insensitivity to dicarboximide fungicides could be a problem with their repeated use, especially since Szejnberg and Jones (1979) showed that dicarboximide insensitivity occurred at high frequencies ($1 \text{ in } 10^5 - 10^6$) in laboratory strains. We detected dicarboximide insensitive isolates in New Zealand orchards in 1985 and Penrose *et al* (1985) reported an Australian case of disease control failure, possibly associated with dicarboximide insensitive *M. fructicola* strains.

Severe limitations may be imposed on the use of fungicides by the presence of insensitive pathogen strains. This paper reports the incidence of insensitivity in *M. fructicola* to dicarboximide and benzimidazole fungicides detected in a survey of the main stonefruit producing regions of New Zealand between March and August 1985. Preliminary results of an investigation of the *in vitro* and *in vivo* fitness of insensitive isolates are also reported.

MATERIALS AND METHODS

Fungicides, media and reference strains

Suspensions of the fungicides vinclozolin (Ronilan FL 500 g ai/litre) and carbendazim (Bavistin FL 500 g ai/litre) were prepared in sterile distilled water. They were diluted with autoclaved potato dextrose agar (PDA) at 50-55 °C and dispensed (20 ml) in plastic petri dishes.

Two dicarboximide sensitive *M. fructicola* strains (PDDCC # 7640, 7642) were used as reference standards in all tests. Subculturing was by mass transfer of conidia and mycelium. In sensitivity tests 6 mm diameter mycelial plugs from the margin of actively growing 6 to 9 day old cultures were placed mycelial surface down on the test medium. All cultures were incubated at 25 °C (± 0.5 °C).

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Survey methods and sensitivity tests

Up to 12 sampling units (usually mummified fruit) were collected randomly in each orchard on a diagonal sampling pattern, and returned to the laboratory. Isolations were made the same day, directly if fresh conidia were present, otherwise after surface sterilisation in 0.5% sodium hypochlorite solution and 70% ethanol. Four replicate samples were plated onto PDA containing 0, 2 or 5 mg ai/litre vinclozolin and 100 mg ai/litre carbendazim. Isolates with colonies greater than 18 mm diameter on 5 mg ai/litre vinclozolin or on 100 mg ai/litre carbendazim after 10 days were classed as insensitive to the respective fungicides.

To distinguish between laboratory and field dicarboximide insensitivity we determined the frequency and level of insensitivity in multiple subcultures of the two reference strains. Field insensitivity was assumed when the frequency obtained from an orchard was significantly greater (by Chi-squared analysis) than that of a laboratory isolate on 5 mg ai/litre vinclozolin (14%).

All isolates (33) with no reduction in growth on 5 mg ai/litre vinclozolin were tested further on 0, 5, 10 and 100 mg ai/litre vinclozolin to determine whether these isolates were insensitive to higher concentrations of fungicide. Four samples of each isolate were tested at each concentration. Growth was determined at 2 day intervals by measuring colony radii, and spore production was assessed. Selected isolates were tested further on 1000 mg ai/litre vinclozolin, on peach (cv. Redhaven) blossoms and on fruit in controlled environments (Elmer and Gaunt unpublished).

RESULTS

Two hundred and thirty nine of the 1292 *M. fructicola* isolates (19%) obtained in the survey grew on PDA containing 5 mg ai/litre vinclozolin (Table 1). The frequency of insensitivity in individual orchards ranged from 0% to 58%. Insensitive isolates were detected at a significantly greater frequency (>22%) than in the laboratory population on three properties in Canterbury, one in Nelson, four in Blenheim, three in Central Otago, and two in Hawkes Bay. Twenty two properties did not have significantly greater frequencies of insensitive isolates, and at seven properties the frequency of insensitivity was significantly less than in the laboratory population. This may reflect disease management strategies or in some cases may be related to sample size. Thirty six isolates (3%) were benzimidazole insensitive, all from the Hawkes Bay region.

TABLE 1: Fungicide insensitivity in isolates of *M. fructicola* from five stonefruit producing areas of New Zealand (1984/85 season).

District	Number of properties assessed	Total number of isolates	Mean frequency of insensitivity in populations			
			vinclozolin %	vinclozolin Range	carbendazim %	carbendazim Range
Central Otago	6	120	21	4-58	0	0
Canterbury	10	296	24	0-48	0	0
Blenheim	9	332	17	6-36	0	0
Nelson	11	376	13	0-28	0	0
Hawkes Bay	6	168	14	5-25	22	0-78

Nine of the 33 vinclozolin insensitive isolates (27%) grew as well on unamended PDA as the sensitive isolates when assessed further on 0, 5, 10, 100 mg ai/litre vinclozolin. One (3%) grew less well and 23 (70%) had larger colonies ($P=0.05$). Spore production varied considerably but the majority (67%) of isolates did not sporulate as well as the sensitive strain ($P=0.05$).

The growth of 11 of the 33 isolates assessed was not reduced on 10 or 100 mg ai/litre vinclozolin ($P=0.05$). Eleven isolates were considered fit and three sporulated profusely. One of these isolates grew well when tested further on 1000 mg ai/litre vinclozolin. This isolate was pathogenic to peach blossoms, sporulated abundantly on infected tissues and caused blossom blight despite a high rate (500 mg ai/litre) of vinclozolin applied prior to pathogen inoculation.

DISCUSSION

Insensitivity to vinclozolin was detected in a significant proportion of orchard isolates of *M. fructicola*. Although discussions with growers indicated no major loss of disease control, one considered that there had been some loss of dicarboximide efficacy after several seasons use. Subsequent investigations at this site revealed that 38% of the isolates were insensitive to some degree on 5 mg ai/litre vinclozolin.

The situation with dicarboximide fungicides appears to differ from that with benzimidazoles, where the presence of insensitive strains led to complete control failures (Beever and Brien 1983). There was a wide range in the degree of dicarboximide insensitivity and there is evidence with other host-pathogen systems that strains with low insensitivity were controlled with full fungicide rates (Hoksbergen and Beever 1984). The majority of insensitive isolates tested *in vitro* grew well but sporulated poorly, and some isolates also grew poorly on fruit. This suggests that they may not be competitive in the absence of dicarboximide fungicides. However, there is cause for concern since several isolates were very insensitive to vinclozolin, grew and sporulated well on unamended media, and were pathogenic to peach flowers and nectarine fruit. Spore production was profuse on affected tissue and blossom blight and fruit rot occurred despite the application of vinclozolin at the highest recommended rate. Vinclozolin, iprodione and procymidone failed to protect nectarines (*Prunus persica* var *nectarina*) against infection and disease development by insensitive isolates (Elmer and Gaunt unpublished).

It is desirable to reduce the selection pressure for dicarboximide insensitive strains of *M. fructicola* in orchards. In most cases of acquired fungicide insensitivity in New Zealand there is a direct relationship between the speed at which insensitivity has appeared and the intensity of fungicide usage (Hartill 1982). Existing brown rot spray programmes should be modified to include non-dicarboximide fungicides to prevent high frequencies of dicarboximide-insensitive strains in the population. In the South Island the re-introduction of a single application of a benzimidazole spray over the bloom period deserves further consideration. However, the use of both dicarboximides and benzimidazoles may be hazardous, particularly in North Island orchards, in view of our laboratory findings that dicarboximide insensitivity may be combined with benzimidazole insensitivity. Several ergosterol biosynthesis inhibiting fungicides have potential for brown rot control, though their use may be limited by a relatively ineffective suppression of sporulation of *M. fructicola* on overwintering sites (Kable 1983).

Successful disease management in the future will depend on an integrated approach to brown rot control. Crop hygiene was neglected on many properties visited. Any practice which decreases the brown rot pathogen population at the beginning of each season may decrease the selection of insensitive strains. In addition cultivars prone to splitting should be avoided. Additional research is required on the fitness of dicarboximide insensitive isolates and the effects of full dicarboximide fungicide rates on low level insensitive *M. fructicola* isolates.

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