

SENSITIVITY OF APPLE BLACK SPOT TO FENARIMOL, FLUSILAZOL, MYCLOBUTANIL, PENCONAZOLE AND DODINE IN NEW ZEALAND

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ABSTRACT

The sensitivity of apple black spot to four DMI fungicides and dodine was evaluated using EC₅₀ and EC₉₅ values for colony growth on amended agar. Sixty-nine orchards with known application histories in the six main apple growing districts of New Zealand were sampled and tested. There was evidence of a shift since 1991 towards populations with reduced sensitivity. No relationship between high EC₅₀ values and spray history was detected, but there was evidence for cross resistance to DMI fungicides. Dodine resistance was not detected. Baseline sensitivities were established for flusilazol and dodine, which had not been used before in resistance tests in New Zealand.

Keywords: *Venturia inaequalis*, DMI fungicides, reduced sensitivity, cross resistance.

INTRODUCTION

Demethylation inhibiting (DMI) fungicides are used increasingly in New Zealand to control apple black spot, caused by *Venturia inaequalis* (Cooke) Winter. Although shifts in resistance have been detected in field isolates in North America (Hildebrand *et al.* 1988), Europe (Fiaccadori *et al.* 1987) and New Zealand (Fowler *et al.* 1990; Whelan *et al.* 1992), the development of field resistance has been less severe and widespread than predicted. In New Zealand, an initial report of resistance (Fowler and Robertson 1990) was confirmed by Whelan *et al.* (1992), who showed a shift towards reduced sensitivity in some strains. Strains resistant to DMI fungicides may arise from mutations to either single or multiple genes (Sholberg *et al.* 1993), and under DMI selection pressure these strains will gradually increase in frequency to a level at which successful control is no longer achieved (Smith *et al.* 1991). Resistance to dodine has not been reported in New Zealand but Sholberg *et al.* (1989) reported some conidia from half of 173 orchards to be resistant to dodine. This paper reports an investigation of the sensitivity of *V. inaequalis* to four DMI fungicides and to dodine in New Zealand in 1994.

MATERIALS AND METHODS

Sixty-nine apple orchards in Auckland, Waikato, Hawkes Bay, Nelson, Canterbury and Central Otago were sampled between January and February 1994. Use of DMI fungicides and dodine during the 1992/93 and the 1993/94 season was recorded for correlation with fungicide sensitivity data. After sampling up to 12 fruit per orchard, conidia were scraped from sporulating lesions and then streaked on water agar (10 g/litre, Germantown) and incubated for 2 to 3 days at 19±1°C with a 12 h photoperiod of fluorescent light. From each isolate ten plugs with germinated conidia were transferred to potato dextrose agar (PDA) plates (39 g/litre, Gibco), amended with benzylpenicillin (100 mg/litre) and streptomycin sulphate (100 mg/litre) to reduce bacterial growth. These were incubated for 2 to 4 weeks at 19±1°C with a 12 h photoperiod of fluorescent light. Two hundred and twenty one isolates were obtained,

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maintained on PDA slopes and tested for resistance to the following products at the concentrations listed:

dodine (technical grade 97%, Rhone-Poulenc, NZ. Ltd) 0.67, 1.33, 2, 2.67 and 5.33 mg/litre
 fenarimol (Rubigan FLO, 120 g/litre, Rhone-Poulenc NZ. Ltd) 0.01, 0.02, 0.05, 0.15 and 0.4 mg/litre
 flusilazol (Nustar, 200 g/kg, DuPont NZ. Ltd) 0.04, 0.08, 0.13, 0.17 and 0.33 mg/litre
 myclobutanil (Systhane 40W, 400 g/kg, Rohm & Haas, NZ. Ltd.) 0.05, 0.1, 0.2, 0.6 and 1.2 mg/litre
 penconazole (Topas 10WP, 100 g/kg, Ciba Geigy, NZ. Ltd.) 0.02, 0.04, 0.07, 0.15 and 0.3 mg/litre

Each isolate was also grown on unamended media as controls. The fenarimol, myclobutanil and penconazole concentrations were based on 1991 tests (Whelan *et al.* 1992). The dodine and flusilazol concentrations were determined in preliminary tests using nine *V. inaequalis* isolates from the 1991 test. Dodine was dissolved in propanol and fenarimol, flusilazol, myclobutanil and penconazole were dissolved in methanol; the final concentrations in the media were less than 0.1%. Fungicide solutions plus 200 mg/litre benzylpenicillin and 200 mg/litre streptomycin sulphate were added to an autoclaved mixture of PDA (19.5 g/litre, Gibco) and water agar (10 g/litre, Germantown). Mycelial plugs (4 mm diameter) from the actively growing margin of colonies of each isolate were transferred to three replicate plates at each fungicide concentration. The plates were incubated at 19±1°C in complete darkness for 28-36 days, after which the diameters of the colonies were measured.

EC₅₀ and EC₉₅ values were calculated from linear regression of the growth and the log₁₀ fungicide concentration. EC₉₅ values followed the pattern of the EC₅₀ values and are therefore not presented in this paper. Resistance factors (Rf) were calculated relative to the lowest values of 1991, to provide a valid basis for comparison. EC₅₀ values of isolates for fungicides to which they had been exposed were correlated with EC₅₀ values of the same isolates for fungicides to which they had not been exposed to estimate the degree of cross resistance selected in the field population. EC₅₀ values of isolates for fungicides to which they had been exposed were also correlated with the numbers of times that these isolates were exposed to the fungicide.

RESULTS

Myclobutanil was used extensively in all regions in 1992/93 and 1993/94. The use of most of the fungicides tested increased in the second season, except for penconazole which decreased in Hawkes Bay and Nelson. Auckland had the lowest overall use of the tested fungicides. The use of DMI fungicides varied widely between orchards,

TABLE 1: Mean EC₅₀ values (mg/litre) of fungicides for *Venturia inaequalis*, sampled from different regions.

Region	fenarimol	penconazole	myclobutanil	flusilazol	dodine
Auckland (6) ¹	0.14(0.082) ² a ³	0.08(0.039)a	0.34(0.255)a	0.07(0.012)ac	0.77(0.032)a
Waikato (6)	0.28(0.197)c	0.11(0.048)ab	0.50(0.279)ab	0.10(0.053)c	0.88(0.087)c
Hawkes Bay (20)	0.19(0.081)ab	0.11(0.042)a	0.48(0.266)b	0.09(0.032)ac	0.82(0.058)b
Nelson (17)	0.21(0.108)bc	0.12(0.048)b	0.45(0.190)ab	0.11(0.047)bc	0.91(0.080)cd
Canterbury (6)	0.15(0.037)a	0.08(0.029)a	0.32(0.089)a	0.07(0.009)a	0.80(0.063)ab
Central Otago (5)	0.15(0.023)ab	0.09(0.015)a	0.31(0.057)ab	0.08(0.015)ac	0.95(0.150)d
All regions (60)	0.19(0.053)	0.10(0.017)	0.43(0.086)	0.09(0.047)	0.85(0.094)

¹ number of orchards tested.

² standard deviations.

³ within each column, mean EC₅₀ values sharing common letters are not significantly different at P<0.05 using LSD-test.

ranging from zero to 12 DMI applications in Nelson in 1992/93 and zero to 11 in Canterbury in 1993/94. The mean number of DMI applications exceeded four in all regions except Auckland in 1992/93. In 1993/94 this number was below four except in Canterbury which had an average of 5.5 DMI applications. The ranges in dodine applications varied from 0 in Auckland to an average of 6.7 in Otago in 1992/93, with a maximum of 18 applications at one property in Nelson in 1992/93.

Sensitivity of *V. inaequalis* to the tested DMI fungicides varied considerably. Both the most sensitive and the most resistant isolates had higher values than in 1991 (Whelan *et al.* 1992). For all DMI products, values of individual isolates were lowest from the Auckland region and highest from the Waikato and Nelson regions. EC₅₀ values of DMI fungicides were lowest for flusilazol and penconazole, and greatest for myclobutanil (Table 1). EC₅₀ values for dodine varied much less: Auckland had the lowest values and Central Otago the highest.

Resistance factors for individual isolates ranged from 3.4 (flusilazol) to 80 (fenarimol) (Table 2). Regional resistance factors were lowest in Canterbury and Otago and highest in Hawkes Bay, Nelson and Waikato. These were greater than in the 1991 tests (Table 2), but much lower than resistance factors of up to 280 times for EC₅₀'s for flusilazol recorded in the USA (Smith *et al.* 1991) where isolates were included that had never been exposed to DMI fungicides. The resistance factors for dodine were much lower than those for the DMI fungicides.

TABLE 2: Comparison of highest and lowest EC₅₀ values and resistance factors (Rf) of the 1991 and 1994 tests (mg/litre).

	fenarimol		myclobutanil		penconazole		flusilazol	dodine
	1994	1991	1994	1991	1994	1991	1994	1994
Highest	1.194	0.186	2.411	1.380	0.299	0.221	0.249	1.442
Lowest	0.065	0.015	0.162	0.045	0.056	0.014	0.067	0.735
Rf-value ¹	79.6	12.4	53.7	30.7	21.4	15.8	3.71	1.96

¹ Rf-values are the highest 1994 values divided by the lowest 1991 values for fenarimol, myclobutanil and penconazole, and by the lowest 1994 values for flusilazol and dodine

EC₅₀ values relative to recommended field application rates were as follows; fenarimol (0.04), myclobutanil (0.05), penconazole (0.015), flusilazol (0.012), dodine (0.07). Cross resistance of isolates to the various DMI's is shown by the high degree of correlation between EC₅₀ values for products to which they were exposed and those to which they were not exposed (Table 3). In the case of myclobutanil, correlations were consistently lower. Myclobutanil has been used most abundantly in recent years, with isolates being highly selected for reduced sensitivity to this product. This could explain lower cross resistance to other DMI fungicides in isolates exposed to myclobutanil.

TABLE 3: Correlation coefficients between EC₅₀ values of *V. inaequalis* for DMI fungicides to which they have been exposed and to which they have not been exposed.

not applied	fenarimol	DMI fungicides applied		
		flusilazol	myclobutanil	penconazole
fenarimol	n.a.	0.929 (69)	0.820 (182)	0.954 (28)
flusilazol	0.993 (8) ¹	n.a.	0.831 (130)	0.957 (36)
myclobutanil	0.997 (5)	0.943 (14)	n.a.	0.988 (6)
penconazole	(0)	0.965 (69)	0.828 (155)	n.a.

¹ Number of samples tested for this combination.

Correlation of EC₅₀ values of isolates for the fungicides they were exposed to with the numbers of times they had been exposed to this fungicide showed no relation between high EC₅₀ values and frequent fungicide use.

DISCUSSION

Black spot samples were taken from orchards with known DMI or dodine application history during the last two seasons, to enhance the probability of detecting reduced sensitivity, and thus this study was not a representative survey of sensitivity. In 1991 isolates were considered to have reduced sensitivity if their value exceeded twice the national mean. Four, five and three isolates were found with high values for fenarimol, myclobutanil and penconazole respectively (Whelan *et al.* 1992). Using the same national mean values to assess the current values, 96%, 47% and 62% of the isolates had reduced sensitivity to fenarimol, myclobutanil and penconazole respectively. Thus both the frequency of the isolates with some reduced sensitivity, and the maximum EC₅₀ values, have increased in the 1994 evaluations compared with 1991.

The highest EC₅₀ values for fenarimol and penconazole were higher than EC₅₀ values found in Italy in 1987 (Fiaccadori *et al.* 1987). The highest EC₅₀ value for flusilazol (0.25 mg/litre) was 1.5 times higher than the highest value found in North America in 1991 (Smith *et al.* 1991). Thus DMI resistance has also increased relative to that measured overseas. No failure to control *V. inaequalis* was reported this season. However, under current management with applications of up to 200% of the recommendations of the New Zealand Committee for Pesticide Resistance, there may be control problems in the near future. It is therefore recommended that development of resistance of apple black spot in New Zealand to DMI fungicides be monitored regularly and that growers and industry be alerted to the fact that current levels of application are associated with a continued increase in resistance.

Dodine resistance was not detected in this study but numbers of applications were high at some properties and some growers have reported apparent control failures. The use of dodine in New Zealand apple orchards has increased considerably over recent seasons with an increased risk of development of resistant populations. This test has established baseline sensitivities of apple black spot to dodine and enables us to monitor future shifts in sensitivity.

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