STRAINS OF *PYRENOPHORA TERES* IN NEW ZEALAND BARLEY CROPS WITH REDUCED SENSITIVITY TO ERGOSTEROL BIOSYNTHESIS INHIBITOR FUNGICIDES

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SUMMARY

Isolates of *Pyrenophora teres* (anamorph *Drechslera teres*) collected from barley crops and fungicide trials during 1986-87 were tested for sensitivity to ergosterol biosynthesis inhibitor (EBI) fungicides. Most isolates from the common 'net' type net blotch (f. *teres*) were very insensitive only to triadimenol whereas most isolates from the 'spot' type (f. *maculata*) were very insensitive to triadimenol and flutriafol and showed markedly reduced sensitivity to imazalil. Some isolates from the 'spot' type were less sensitive to prochloraz than isolates of the 'net' type.

INTRODUCTION

The introduction of ergosterol biosynthesis inhibitor (EBI) fungicides in the late 1970s was hailed as a major breakthrough because they were a group of compounds with diverse chemical structures which controlled a wide range of diseases, and field resistance was thought unlikely to become a problem (Fuchs and Drandarevski 1976; Siegel 1981). The first observation of possible field resistance was made in 1981-82 when triadimenol-containing seed treatments failed to control net blotch caused by *Pyrenophora teres* Drechsler (anamorph *Drechslera teres* (Sacc.) Shoemaker) in some New Zealand barley crops (Sheridan and Grbavac 1982). Subsequent studies confirmed that failure was due to resistance to triadimenol in the net blotch pathogen (Sheridan *et al* 1985). Cross resistance to nuarimol was common, but cross resistance to imazalil, prochloraz and propiconazole was not detected (Sheridan *et al* 1987).

Up to the 1985-86 season only the typical 'net' type of net blotch (*P. teres* Drechs. f. *teres*) had been identified in New Zealand. In January 1986 the 'spot' type (*P. teres* Drechs. f. *maculata* Smedg.) (Smedegaard-Petersen 1971) was identified from the South Island and subsequently from the North Island. It seemed that the currently used EBI fungicides did not give adequate control of the 'spot' type (Sheridan, Grbavac unpub.). The 'spot' type has been reported from Canada and Israel (1967), Norway (1969), Denmark (1971), Finland (1972) and Australia (Khan and Tekauz 1982) but little is known about its sensitivity to EBI's. This paper reports the results of laboratory tests for sensitivity of isolates of both types to EBI fungicides using the poison plate method.

MATERIALS AND METHODS

Source of isolates: Collections of barley leaves with net blotch symptoms were made during the annual cereal disease survey in the North Island in 1986-87 and some specimens were received from colleagues. Further collections were made from barley plants following one fungicide spray application in trials at Ahiaruhe in the Wairarapa. Both the 'net' and 'spot' type were present in these trials. After harvest, collections were made from infected barley regrowth.

Infected leaf pieces were placed onto malt extract agar amended with 10 µg/ml benomyl (MEA + B) to suppress saprophytic fungi and incubated for up to 5 days under NUV light on the laboratory bench. Mycelial growth was transferred to further plates of MEA + B to establish pure cultures and identity was confirmed by the presence of conidia of *D. teres*.

Standard cultures for comparison comprised one from seed cv. Carlsberg harvested in New Zealand in 1976 and stored in a deep freeze until 1986, and one from seed of ANT-148 imported from Denmark in 1984. An isolate, showing reduced sensitivity to some EBI's, from the 'spot' type occurring on plants of cv Kym collected from the

Proc. 40th N.Z. Weed and Pest Control Conf.

South Island in January 1986 was also included in some tests.

Sensitivity testing: The fungicides were added directly to malt extract agar medium contained in 500 ml Duran bottles before sterilization. Plates were poured to contain approx 20 ml when the medium had cooled to about 50 °C. Plugs of actively growing mycelium 5 mm in diameter were transferred to plates of MEA amended with EBI fungicide (Table 1). Plates were incubated on the laboratory bench and colony diameter measured to the nearest mm at intervals.

TABLE 1: Fungicides used in the poison plate sensitivity tests of *Drechslera teres* isolates.

Common name and year introduced	EBI class	Product name, formulation and supplier
flutriafol 1983	triazole	Impact 12.5% EC. ICI
imazalil 1972	imidazole	Fungaflor 75% WP. NZFF
prochloraz 1977	imidazole	Sportak 45% EC. NZFF
propiconazole 1979	triazole	Tilt 25% EC. Ciba-Geigy
triadimenol + fuberidazole 1977	triazole	Baytan F17 15 + 2% WP Bayer
triadimenol + imazalil —	triazole + imidazole	Baytan IM. 15 + 5% WP Bayer

TABLE 2: Mean colony diameter* of *Drechslera teres* isolates on media amended with EBI fungicides.

Isolate Source		Fungicide concentration (µg/ml)				
		0	triadimenol + imazalil 30 + 10	triadimenol 50	imazalil 10	propiconazole 10
FARMERS CROPS 1986†						
'Net' type	1 49	0	16	7	0	3
	2 55	0	21	3	0	3
	3 49	0	0	13	0	0
	4 24	0	6	0	0	0
	5 55	0	13	5	0	0
	6 56	0	19	12	0	9
	7 53	0	13	5	0	0
	8 57	0	17	5	0	0
	9 61	3	17	4	0	0
	10 57	0	13	5	2	0
	11 56	0	0	5	2	0
	12 54	0	15	5	0	0
'Spot' type	13 51	10	17	19	1	8
	14 48	12	30	17	1	10
STANDARDS						
N.Z. Carlsberg 1976††	48	0	0	12	2	8
N.Z. Kym 1986†††	66	9	33	18	0	12
Danish ANT 1984	52	9	26	15	6	11

* diameter in mm less 5 mm for inoculum plug for 2 reps after 7 days incubation at 19-26 °C.

† from triadimenol + imazalil treated seed of cv Triumph except No. 4 which is untreated Zephyr.

Nos. 1-4 from the Wairarapa, 5-12 from the Manawatu and 13-14 from Nelson.

†† 'net' type

††† 'spot' type

RESULTS

Sensitivity of isolates from farmers crops. Most isolates of the 'net' type grew on media amended with 50 µg/ml triadimenol producing about 30% of the growth obtained on non-amended medium (Table 2). Two of the isolates were as sensitive to triadimenol as the 1976 standard isolate. Most isolates grew on 10 µg/ml imazalil but only a small number of isolates produced any growth on 10 µg/ml propiconazole or prochloraz.

The two isolates of the 'spot' type from the Nelson district of the South Island produced greater growth on imazalil, triadimenol + imazalil and prochloraz than did any isolate of the 'net' type.

Sensitivity of 'net' and 'spot' type isolates. All of the 'spot' type isolates from barley regrowth grew on medium containing 100 µg/ml triadimenol and flutriafol, most (71%) on this concentration of imazalil and some (29%) on this concentration of prochloraz (Table 3). None grew on 100 µg/ml propiconazole. No isolate of the 'net' type grew on 100 µg/ml of any EBI tested except triadimenol. In another test some isolates of the 'net' type grew well on 50 µg/ml flutriafol.

Effect of application of EBI fungicide sprays on sensitivity. No marked change in sensitivity to any EBI was detected (Table 4).

TABLE 3: Percentage 'spot' and 'net' type isolates of *Drechslera teres* growing on media amended with EBI fungicides*. Mean diameter growth in mm is given in brackets.

Isolate source and number tested	Fungicide concentration (µg/ml)					
	0	triadimenol 100	imazalil 100	propiconazole 100	prochloraz 100	flutriafol 100
BARLEY REGROWTH 1987						
'Net' type 7†	100(55)	57(2)	0	0	0	0
9††	100(59)	56(4)	0	0	0	0
'Spot' type 7†	100(64)	100(20)	71(4)	0	29(1)	100(14)

* 2 reps/isolate after 10 days growth at 15-24 °C

† fungicide spray trial

†† farmers crops

TABLE 4: Mean colony diameter* of *Drechslera teres* isolates on media amended with EBI fungicides.

Isolate source and number tested	Fungicide concentration (µg/ml)											
	0	triadimenol			imazalil		propiconazole		prochloraz		flutriafol	
		10	50	100	10	50	10	50	10	50	10	50 100
BARLEY REGROWTH 1987†												
'Spot' type												
ex-												
propiconazole	5	42	47	25	13	7	5	5	<1	3	0	38 12 9
prochloraz	5	49	54	25	17	6	4	5	<1	5	0	37 20 13
flutriafol	5	43	42	21	10	9	4	17	1	4	0	28 16 7
triadimenol/												
tridemorph ¹	5	37	47	25	15	7	4	6	<1	7	0	35 21 11
untreated	5	47	32	22	10	7	2	17	<1	7	<1	26 15 8

* diameter in mm less 5 mm for inoculum plug for 2 reps after 10 days at 9-21 °C.

† collections made from fungicide spray trial plots 3 weeks after harvest: March 1987.

¹ Bayfidan M

DISCUSSION AND CONCLUSIONS

Most isolates of the 'spot' type of net blotch were very insensitive to triadimenol and flutriafol and had reduced sensitivity to imazalil. No isolate of the 'net' type grew at 100 µg/ml flutriafol, imazalil or prochloraz whereas 100%, 71% and 29% respectively of the 'spot' type grew.

No evidence of reduced sensitivity to EBI fungicides, other than to triadimenol and flutriafol, was detected in isolates of the 'net' type supporting earlier findings. Cross resistance between triadimenol, flutriafol and nuarimol appears to be common in both types (Kendall 1986; Sheridan *et al* 1987). Sensitivity to propiconazole appeared to be similar for both types of net blotch.

The most popular seed treatment for barley at the present time is 'Baytan IM' containing triadimenol + imazalil. The presence in New Zealand of strains of *D. teres* f. *maculata* with reduced sensitivity to these EBI's could jeopardise the efficacy of seed treatments and foliar sprays in current use. These strains were not found in farmers crops in the North Island but appear to be not uncommon in the South Island. A survey of barley crops in the South Island should be carried out to obtain reliable data on the prevalence of the 'spot' type.

It is unlikely that 'spot' type strains with reduced sensitivity to EBI fungicides developed in this country because the 'spot' type was not found here until January 1986. The source of triadimenol resistant strains of the 'net' type was traced to seed from overseas (Sheridan *et al* 1985). The 'spot' type may also have originated overseas because the range and level of sensitivity to EBI fungicides is very similar to that of the Danish isolate.

ACKNOWLEDGEMENTS

We are grateful for the co-operation and assistance of the chemical industry and farmers during the course of these studies.

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