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DMI sensitivity and cross-resistance patterns of Rhynchosporium secalis isolates from South Africa

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Abstract

Isolates of R. secalis were collected yearly from the Ruens area of the Western Cape during the 1993–1995 growing seasons. These isolates were evaluated in vitro to determine sensitivity to triazole fungicides (triadimenol, tebuconazole, usilazole and propiconazole). The sensitivity fluctuated but in 1995 isolates were significantly less sensitive towards triadimenol than in the previous two years. In a second experiment, isolates collected from two fields with a 5–6 year history of triadimenol seed treatments and tebuconazole applications were evaluated for their fungicide sensitivity. A significant positive correlation was observed between tebuconazole and triadimenol sensitivity among R. secalis isolates from these fields. However, such a correlation was not found within the R. secalis population collected during 1993–1995 where shorter crop rotation patterns and a range of fungicides were applied. In a third experiment, the fungicide sensitivity of local R. secalis isolates was evaluated towards two new triazole fungicides, namely bromuconazole and triticonazole. Correlation coefficients observed between these new triazoles and those previously applied in South Africa were not significantly positive. The lack of significant cross-resistance has important practical implications for management of fungicide resistance.

Keywords: Rhynchosporium secalis; Fungicide sensitivity; Monitoring

1. Introduction

Barley scald, caused by Rhynchosporium secalis (Oud) J. Davis, is the most serious disease of barley in South Africa, with yield losses being mainly attributed to a reduction in 1000-kernel weight (Khan and Crosbie, 1988; Scott et al., 1992). In South Africa, a yield increase of 37% was achieved after plants of the susceptible cultivar Clipper were sprayed with two applications of the triazole fungicide, propiconazole (Scott et al., 1992). Triazole fungicides inhibit the C14 demethylation step in fungal ergosterol biosynthesis and are referred to as demethylation inhibitors (DMIs) (Copping et al., 1984). It is generally accepted that a small population of resistant genotypes occur naturally in pathogen populations before the first fungicide applications (Brent, 1992). Under selection pressure from the triazole fungicides, however, the fungal population can shift towards reduced sensitivity, and the proportion of resistant phenotypes may reach a level where satisfactory disease control is no longer achieved (Brent, 1992). This leads to the development of practical resistance.

Cross-resistance studies are useful in assessing the risk of resistance development. Cross-resistance to fungicides has been defined as resistance to two or more fungicides as a result of the same genetic factor (Georgopoulos, 1977). For Pyrenophora teres populations, it has been reported that correlation coefficients of resistance to DMIs may differ between populations within the species (Peever and Milgroom, 1993). A change in fungicide sensitivity involves changes in the frequencies of genes controlling fungicide resistance in the pathogen population. Fungal genes may control resistance to one or two fungicides and depending on their frequency will influence the fungicide management strategy to be followed.

Triadimenol has been used in South Africa since 1979 as a seed treatment for barley scald control. Foliar sprays

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such as propiconazole, usilazole and tebuconazole were introduced during 1984, 1988 and 1989, respectively. Although the triazoles have been used extensively for almost two decades in South Africa, no information is available on the sensitivity and cross-resistance patterns of the Rhynchosporium secalis population to this fungicide class. The aim of this study was to determine the sensitivity and cross-resistance patterns of the R. secalis population against triadimenol, propiconazole, usilazole, tebuconazole and two recently introduced triazoles, bromuconazole and triticonazole.

2. Materials and methods

Three experiments were conducted on R. secalis isolates from the Ruens area of the Western Cape, which is the main area where malting barley is grown in South Africa. In the rst experiment, isolates were screened against four commonly used triazoles, namely triadimenol, propiconazole, usilazole and tebuconazole. In the second experiment, two elds were selected with a longer history of fungicide usage (5 6 years). Isolates from these elds were screened against the commonly used fungicides, namely triadimenol and tebuconazole. In the third experiment, isolates were screened against two triazoles not previously used in the Western Cape, namely bromuconazole and triticonazole. The minimum inhibitory concentration (MIC) of each fungicide was determined for each isolate. Isolates from the Ruens were also compared with the fungicide sensitivity of wild-type R. secalis isolates. The term wild-type is an arbitrary designation for one or more strains chosen deliberately as genetic standards (Yoder et al., 1986). The R. secalis population in the Ruens has been continuously subjected to fungicide applications. It was, therefore, decided to collect isolates from fodder barley growing in the Swartland area, as this crop had not yet been subjected to fungicide applications and wheat elds largely dominate the area. Subsequently these R. secalis isolates were considered as being unselected by fungicides and a comparison with wild-type isolates from Australia (kindly provided by H. Wallwork) and England (kindly provided by S. Kendall) showed that they had similar levels of fungicide sensitivity. These wild-type isolates were also considered as indicative of the base-line sensitivity.

2.1. Isolation and in vitro fungicide sensitivity assay

Leaf segments with scald lesions were surface sterilised, rinsed in sterile water, placed on moist lter paper in Petri dishes and incubated for two days at 173C in the dark. Spores that developed on lesion surfaces were dislodged with a sterile scalpel. Spores were then transferred to water agar plates supplemented with streptomycin (0.05 g streptomycin sulphate/L) and incubated for 24 h, after which time germinating single spores were transferred to lima bean agar (LBA) (62 g lima beans, 12 g Biolab agar/l). Single spore colonies were subsequently cultured on LBA and stored on malt extract agar (20 g malt extract, 12 g Biolab agar/l) slants under sterile mineral oil at 33C in the dark.

For fungicide sensitivity testing, sterile LBA was amended with technical grade fungicide. The technical grade fungicides were dissolved in a 70% ethanol solution to create a stock solution of 4000 l g ai/ml. Fungicide concentrations of 0, 0.03, 0.1, 0.3, 1, 3, 10, 30 and 60 l g ai/ml were prepared by dilution of the stock solution into autoclaved LBA after being cooled to 503C. Control plates consisted of LBA and ethanol (which never exceeded 1.1%). Medium was poured into Petri dishes (90 mm diam.), and a mycelial disk (3 mm diam.) cut from the edge of an actively growing culture with a cork borer, inverted and placed in the centre of each plate. Each isolate was tested on nine concentrations of each fungicide. Three isolates of known sensitivity were included with each set of tests to provide a check. Fungal growth was assessed after 14 days of incubation at 173C in the dark, and the minimum fungicide concentration required to inhibit growth (MIC) recorded.

2.2. Statistical analysis

All MIC values were transformed to log scale so as to normalise the observed variation as required by the analysis of variance and by the Pearson correlation coe$ cient. In the case of the wild-types hardly any variation was observed; F-values for comparisons involving the wild-types were therefore obtained by squaring Cochran s t-like statistic (Snedecor and Cochran, 1989, p. 97) involving two error mean squares: Error(a) variance within wild-types; Error(b) variance within other types.

2.3. Sensitivity towards triadimenol, propiconazole, yusilazole and tebuconazole

Rhynchosporium secalis isolates (50 isolates per year) collected during 1993 (12 elds), 1994 (12 elds) and 1995 (14 elds) were tested for their fungicide sensitivity to triadimenol, propiconazole, usilazole and tebuconazole, which have been used frequently in the Western Cape (Table 1). All isolates were tested simultaneously towards each of the respective fungicides. Most samples (approximately 60%) were taken from elds where barley had been grown the previous year. These elds were not selected according to the amount of fungicides sprayed but randomly although with a bias towards barley production in previous years. Two to seven single spore isolates per eld were evaluated to determine their fungicide sensitivity. Replicate samples from elds varied and the distance between samples varied from being in the

Table 1
Information regarding the Rhynchosporium secalis isolates evaluated in the rst and third experiment

Locality	District	No. of isolates in rst experiment	No. of isolates in third experiment	Year of isolation
3	Riversdal	5		1993
4	Caledon	5	2	1993
5	Swellendam	5	2	1993
6	Bredasdorp	7	2	1993
7	Caledon	5	2	1993
8	Bredasdorp	5	2	1993
9	Bredasdorp	5		1993
10	Bredasdorp	5	1	1993
11	Caledon	2	1	1993
12	Heidelberg	3	1	1993
13	Heidelberg	3		1993
14	Swellendam	2	1	1993
15	Riversdal	5	1	1994
16	Caledon	7	4	1994
17	Bredasdorp	6	2	1994
18	Caledon	6	1	1994
19	Bredasdorp	3		1994
20	Caledon	3		1994
21	Bredasdorp	3		1994
22	Bredasdorp	8	1	1994
23	Bredasdorp	8	1	1994
24	Caledon	7	1	1994
25	Caledon	7	1	1994
26	Caledon	5		1994
27	Caledon	6	2	1995
28	Caledon	5	3	1995
29	Caledon	5	3	1995
31	Caledon	7		1995
32	Caledon	5		1995
33	Caledon	7		1995
34	Caledon	3		1995
36	Caledon	6		1995
37	Caledon	6		1995
38	Caledon	5	2	1995
39	Heidelberg	6	2	1995
40	Swellendam	6		1995
42	Caledon	7	1	1995
43	Caledon	7	4	1995

same lesion to 25 m apart within each eld. In total 150 isolates were randomly collected from 36 di erent barley elds scattered throughout the Ru(ens in the Western Cape. Fungicide sensitivity of isolates collected during 1993 1995 was compared with each other and with wild-type isolates.

2.4. Sensitivity of R. secalis isolates from xelds with continuous triadimenol and tebuconazole applications

In order to evaluate the fungicide sensitivity of R. secalis populations subjected to continuous triazole applications, two commercial barley elds with known fungicide histories were chosen as collection sites in the Bredasdorp district. At locality A, tebuconazole was applied from 1991 to 1996, with the spray dosage varying from 150 to 175 g/ha. Additionally, seed was treated with triadimenol from 1991 to 1994 and with triticonazole in 1995 and 1996. At locality B, the spray dosage of tebuconazole varied from 187.5 to 271 g/ha during 1992 1996, and seed was treated with triadimenol for 3 years (1992 1994), and with triticonazole the following two years (1995 1996). The dosage given for spray applications were represented by one tebuconazole spray per season. Within each eld 7 locations were sampled, and ultimately 41 single spored isolates from locality A, and 45 single spored isolates from locality B were evaluated for their sensitivity towards tebuconazole and triadimenol. The distance between samples within location A and within location B varied from being in the same lesion to 50 m apart. Fungicide sensitivity of isolates from these two elds was compared with that of isolates from the rst experiment and with wild-type isolates by means of an analysis of variance. Cross-resistance between tebuconazole and triadimenol was determined with Pearson s correlation analysis.

2.5. Sensitivity of isolates to bromuconazole and triticonazole and cross-resistance

Two triazoles, namely bromuconazole and triticonazole were registered for use against R. secalis in the Western Cape during 1995. Eighty R. secalis isolates collected prior to the widespread use of these fungicides in the area (prior to 1996) were subsequently tested for their sensitivity towards these fungicides and compared with wild-type R. secalis isolates. Cross-resistance patterns between bromuconazole and triticonazole and other triazoles previously used in the Western Cape were determined with Pearson s correlation analysis.

3. Results

Obtaining great numbers of R. secalis isolates from fodder barley cultivars, to represent the wild-type population, proved di$ cult because of their resistance to R. secalis. Consequently only 11 wild-type isolates were collected from a fodder barley eld for use in this study. This small number of isolates is probably not fully representative of the wild-type population.

3.1. Sensitivity towards triadimenol, propiconazole, yusilazole and tebuconazole

Average MIC values of R. secalis isolates collected in the Cape Ru(ens in 1993 were 9.8, 17.5, 2.6 and 3.11 g/ml when tested against triadimenol, propiconazole usilazole and tebuconazole respectively. Average MIC values of R. secalis isolates collected in 1994 were 12.7, 11, 3.7 and 3.71 g/ml when tested against triadimenol,

propiconazole, usilazole and tebuconazole, respectively. Average MIC values of R. secalis isolates collected in 1995 were 18.8, 7.9, 1.1 and 2.9 l g/ml when tested against triadimenol, propiconazole, usilazole and tebuconazole, respectively. In comparison, the MIC values of the wild-type isolates towards the same fungicides were 1.2, 0.8, 0.3 and 0.4 l g/ml, respectively. The fungicide sensitivity of R. secalis isolates collected during the 1993 1995 seasons was signi cantly di erent from that of wild-type isolates regarding the four triazoles evaluated (Tables 2 5). There were also signi cant di erences in sensitivity towards usilazole between isolates collected from di erent years (Table 3). In general the sensitivity uctuated, but in 1995 isolates were signi cantly less sensitive towards triadimenol than in the previous two years (Tables 2, 4 and 5).

3.2. Sensitivity of R. secalis isolates from xelds with continuous triadimenol and tebuconazole applications

Average MIC values of isolates from locality A and B were respectively 13.95 and 17.82 l g/ml when tested towards triadimenol. The triadimenol sensitivity of isolates from both elds were not signi cantly di erent from each other and not di erent to the triadimenol sensitivity found in the 1993 1995 isolates (Table 4). Isolates from locality A and B evaluated against tebuconazole had average MIC values of 3.72 and 4.96 l g/ml, respectively. The tebuconazole sensitivity of isolates from both elds were not signi cantly di erent from each other but di ered signi cantly from the sensitivity observed in the 1993 1995 isolates (Table 5). Wild-type R. secalis isolates evaluated against triadimenol and tebuconazole had average MIC values of 1.2 and 0.4 l g/ml, respectively. R. secalis isolates from elds A and B were signi cantly di erent in their sensitivity towards triadimenol and tebuconazole compared to wild-type isolates (Tables 4 and 5). The correlation coe$ cients between triadimenol

Table 2
Analysis of variance of in vitro sensitivity of R. secalis isolates towards propiconazole

Source	df	Sum of squares	Mean square	F-value	P
Between all types	3	55.2048	18.4016		
Wild-type vs. rest	1	43.1740	43.1740	102.2018	0.0000
1993, 94 vs. 95	1	11.3981	11.3981	8.66	0.0038
1993 vs. 1994	1	0.6024	0.6024	0.46	0.4997
Error(a)	8	2.8991	0.3624		
Error(b)	148	202.4620	1.3680		
Total	159	260.5659			

F square of Cochran s t-like statistic.

Table 3
Analysis of variance of in vitro sensitivity of R. secalis isolates towards usilazole

Source	df	Sum of squares	Mean square	F-value	P
Between all types	3	56.7560	18.9187		
Wild-type vs. rest	1	20.5882	20.5882	326.1790	0.0000
1993, 94 vs. 95	1	32.3752	32.3752	39.00	0.0001
1993 vs. 1994	1	3.664	3.664	4.41	0.0373
Error(a)	8	0	0		
Error(b)	146	127.8302	0.8755		
Total	157				

F square of Cochran s t-like statistic.

Table 4
Analysis of variance of in vitro sensitivity of R. secalis isolates towards triadimenol

Source	df	Sum of squares	Mean square error	F-value	P
Between all types	5	54.2381	10.8476		
Wild-type vs. rest	1	46.3822	46.3822	310.6077	0.0000
Fields vs. Years	1	1.5562	1.5562	2.9856	0.0854
Field A vs. B	1	1.2671	1.2671	2.4311	0.1203
1993, 94 vs. 95	1	3.9421	3.9421	7.5632	0.0064
1993 vs. 1994	1	1.1483	1.1483	2.2031	0.1391
Error(a)	8	1.0728	0.1341		
Error(b)	227	118.3158	0.5212		
Total	240	173.6267			

F square of Cochran s t-like statistic.

Table 5
Analysis of variance of in vitro sensitivity of R. secalis isolates towards tebuconazole

Source	df	Sum of squares	Mean square error	F-value	P
Between all types	5	76.7265	15.3453		
Wild-type vs. rest	1	38.4384	38.4384	119.4241	0.0000
Fields vs. Years	1	18.4045	18.4045	15.7675	0.0001
Field A vs. B	1	2.9902	2.9902	2.5617	0.1108
1993, 94 vs. 95	1	12.7006	12.7006	10.8809	0.0011
1993 vs. 1994	1	3.7048	3.7048	3.1739	0.0761
Error(a)	10	0.5073	0.0507		
Error(b)	240	280.1387	1.1672		
Total	255	359.5207			

F square of Cochran s t-like statistic.

Table 6
Patterns of cross-resistance to triadimenol and tebuconazole in Rhynchosporium secalis populations from localities with 5 6 years of continuous fungicide applications, as indicated by the Pearson correlation coe$ cients

Locality	A (n 41)	B (n 45)
r	0.64340	0.51783
P	0.0003	0.0001

Number of observations.
Correlation coe$ cient.
Signi cance level of r.

and tebuconazole analysed from both localities were signi cantly positive (Table 6).

3.3. Sensitivity towards bromuconazole and triticonazole and cross-resistance patterns

Signi cant positive correlation coe$ cients were found between bromuconazole and three other fungicides namely triticonazole, tebuconazole and usilazole (Table 7). Signi cant positive correlation coe$ cients were observed between triticonazole and two other triazoles, tebuconazole and usilazole. The correlation coe$ cients between both bromuconazole and triticonazole and the two remaining fungicides in the study, triadimenol and propiconazole were respectively not signi cant (Table 7).

Table 7
Patterns of cross-resistance to demethylation inhibitor (DMI) fungicides in the Rhynchosporium secalis population from the Ruens area, as indicated by the Pearson correlation coe$ cients

Fungicide	Bromuconazole	Triticonazole
Triticonazole		
r	0.57449	
P	0.0001	
n	78	
Triadimenol		
r	0.07097	0.02332
P	0.5424	0.8342
n	76	83
Tebuconazole		
r	0.34014	0.44380
P	0.0023	0.0001
n	78	86
Flusilazole		
r	0.38517	0.32957
P	0.0008	0.0028
n	73	80
Propiconazole		
r	0.15849	0.15799
P	0.2524	0.2125
n	54	64

Correlation coe$ cient.
Signi cance level of r.
Number of observations.

Wild-type R. secalis isolates had average MIC values of 0.77 and 1.36 l g/ml when tested against triticonazole and bromuconazole, respectively. R. secalis isolates differed signi cantly in their triticonazole (Cochran s t 2.6058; P 0.0179) and bromuconazole (Cochran s t 8.3859; P 0.0000) sensitivity compared to the wild-type isolates.

4. Discussion

The triazole sensitivity of R. secalis isolates collected during the 1993 1995 seasons uctuated, showing no trend towards resistance build-up except towards triadimenol. In some cases, R. secalis isolates from 1995 were signi cantly more sensitive towards tebuconazole, propiconazole and usilazole than in the previous years (Table 8). Isolates collected in 1995 were signi cantly less sensitive than isolates collected during the previous two years (Fig. 1, Table 8). The apparent uctuation in sensitivity of isolates towards these triazoles could be explained by the several factors. Triadimenol is the active ingredient of Bavistin a seed treatment introduced in 1979 which dominated the market for years and was widely applied in the Western Cape. This exerted an enormous selection pressure on the R. secalis population. However, a wide range of triazole fungicides was available as foliar applications to control scald, which limited the selection pressure exerted by the foliar application of a speci c triazole. A correlation analysis (Table 9) supported this theory and showed that no signi cant positive correlation (P 0.05) between triadimenol and the other triazoles tested in this study occurred in the R. secalis population collected from 36 di erent elds during the 1993 1995 period. The lack of triadimenol cross-resistance in this population, use of di erent triazoles as foliar applications and crop rotation practices, possibly resulted in a uctuating sensitivity of the population towards usilazole, propiconazole and tebuconazole. However, as triadimenol was continuously applied throughout these elds, the shift detected towards this fungicide may be due to an evolutionary process.

In the second experiment investigating the e ect of continuous fungicide use on fungicide sensitivity and cross-resistance patterns, however, it was evident that a signi cant positive correlation in sensitivity towards triadimenol and tebuconazole can develop (Table 6). These ndings are in accordance with the observations made by Peever and Milgroom (1993) regarding the DMI sensitivity of Pyrenophora teres, namely that correlation relationships between DMIs di er among populations. It seems that correlation relationships can also di er among populations of R. secalis. The two elds from which isolates were collected for the second experiment are situated in an area where farmers have

Table 8
Mean log MIC values and standard errors of R. secalis isolates evaluated for their in vitro sensitivity against four triazole fungicides in the rst experiment

Source		Triadimenol	Tebuconazole	Propiconazole	Flusilazole
1993	Mean	2.14	0.49	2.12	0.49
	Standard error	0.086	0.161	0.191	0.139
1994	Mean	2.36	0.87	1.97	0.88
	Standard error	0.094	0.127	0.136	0.137
1995	Mean	2.6	0.1	1.46	0.30
	Standard error	0.130	0.181	0.162	0.123
Wild-types	Mean	0.12	1.19	0.40	1.2
	Standard error	0.122	0.155	0.201	0

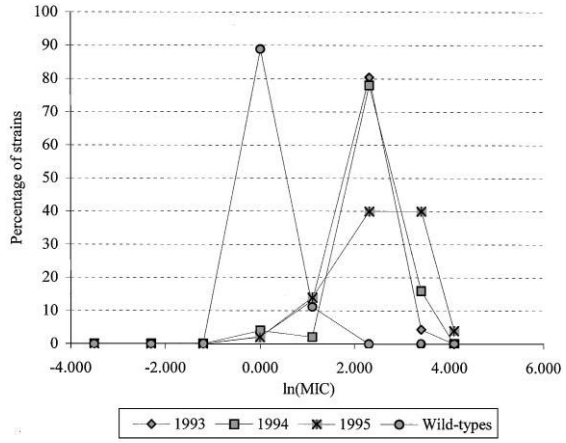

Fig. 1. Changes in sensitivity of Rhynchosporium secalis to triadimenol since 1993, and a comparison with wild-type isolates

Table 9
Patterns of cross-resistance between triadimenol and three triazoles, tebuconazole, propiconazole and usilazole in Rhynchosporium secalis populations collected during the 1993 1995 period, as indicated by the Pearson correlation coe$ cients

	Tebuconazole	Propiconazole	Flusilazole
r	0.07097	0.00107	0.08414
P	0.4290	0.9914	0.3509
n	126	105	125

Correlation coe$ cient.
Signi cance level of r.
Number of observations.

a rotation pattern with longer periods of barley production (5 6 years). Thus, longer periods of fungicide application occur than usually found in the Rutens area (2 3 years). Under this selection pressure one would expect selection of genes conveying a reduced sensitivity to both fungicides. This also suggests that the frequency of genes which control resistance to both triadimenol and tebuconazole is high, and seems similar to the population in the study by Kendall et al. (1993).

Cross-resistance has important practical implications for management of resistance. Uncorrelated coe$ cients suggest that di erent genetic factors control resistance to each DMI. No signi cant correlation coe$ cients indicate that no relation exists between these triazoles in a particular population and therefore no tendency of MIC values to increase together. These ndings suggest, therefore, that combining or alternating these triazoles would be useful in controlling disease and managing resistance build-up in South African barley elds.

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