

## Evaluation of fungicides to control take-all and rhizoctonia root rot of wheat

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### Summary

A series of experiments was undertaken to determine the effect of seven fungicides (at potentially commercial rates) on the *in vitro* growth of *Gaeumannomyces graminis* var. *tritici* (Ggt) and *Rhizoctonia solani* (AG-8) and on take-all and rhizoctonia root rot of wheat. Hexaconazole, flusilazole, prochloraz and propiconazole were very effective in reducing growth of Ggt, whereas all seven reduced the growth of *R. solani*. Similarly, in the pot experiment, only mepronil reduced take-all of wheat, whereas all seven fungicides reduced the severity of rhizoctonia root rot. Of the fungicides tested in a micro-plot experiment, hexaconazole, prochloraz and propiconazole reduced take-all (flusilazole was also tested) and all four reduced rhizoctonia root rot. Prochloraz and hexaconazole increased seed weight in take-all infected plants, but none of the fungicides had any effect on yield of plants infected with rhizoctonia root rot. In the absence of either disease, the fungicides had little or no effect on plant growth.

### Introduction

Take-all and rhizoctonia root rot [caused by *Gaeumannomyces graminis* (Sacc.) von Arx and Olivier var. *tritici* Walker and *Rhizoctonia solani* Kuhn, respectively] are the most serious fungal root diseases of wheat (*Triticum aestivum* L.) in southern Australia. They may occur together, particularly in the light calcareous sandy loams characteristic of the Mallee regions of South Australia, Victoria and Western Australia (Rovira and Venn 1985, R.F. de Boer personal communication, Cotterill 1991) and other light sandy soils (MacNish 1988). Although several measures are available for the control of take-all, such as the use of non-cereal rotation crops, control of grass weeds and the use of nitrogenous fertilizers, particularly  $\text{NH}_4\text{-N}$  sources (MacNish 1988), there are few options available for the control of rhizoctonia root rot. The most effective control measure for the latter disease is considered to be cultivation (Jarvis and Brennan 1986, Rovira 1986, de Boer and Kollmorgen 1988), however this may exacerbate take-all (MacNish 1985, Cotterill

and Sivasithamparam 1988) and cause greater soil erosion. The use of fungicides is one means of control which could possibly overcome this problem.

Chemical control of take-all of wheat has been achieved with fungicide-amended fertilizers applied to the drill row at seeding (Ballinger and Kollmorgen 1988). Two fungicides, both ergosterol-inhibiting triazole compounds, have been registered; flutriafol at 100 and triadimefon at 200 g a.i.  $\text{ha}^{-1}$ . Neither compound has proved to be very effective in reducing rhizoctonia root rot and increasing wheat yields in field situations, with other triazoles such as tebuconazole (Cotterill 1990, 1991) and diniconazole (Cotterill 1991) outperforming them. If the risk of infection from two or more soil-borne pathogens exists in certain situations, then the use of a fungicide which selectively controls only one of them may result in little improvement in yield, as Murray (1988) has suggested. Although very similar in structure, the triazole fungicides vary quite markedly in their effects on the two diseases, for example, the ability of tebuconazole (Bayer HWG 1608) to reduce rhizoctonia root rot (Cotterill *et al.* 1989, Cotterill 1990) was not matched by its performance against take-all in a pot experiment (Cotterill *et al.* 1992).

The aim of this study was to examine the performance of seven fungicides against take-all and rhizoctonia root rot of wheat under the same environmental and edaphic conditions.

### Materials and methods

#### Fungal isolates and inoculum

Three isolates of each fungus were used throughout the study. Ggt isolates 84.8, Mr 9.5 and Mr 9.6 were collected from a wheat field at Walpeup, Victoria, and the three *R. solani* (AG-8) isolates 194, 204 and 447 from Woomelang, Victoria.

For each pathogen, colonized millet inoculum (used in experiment 1 and the micro-plot experiment) was prepared by soaking 1 L Japanese barnyard millet [*Echinochloa frumentacea* (Roxb.) Link] in a 2 L glass flagon overnight, draining off the excess water and then autoclaving at 100 kPa for 45 min, on three consecutive days. A piece of 20% potato dextrose agar (PDA) colonized with *R. solani* was placed on the moist millet and the flagon resealed and incubated for 28 days at 25°C. The colonized millet was dried in a laminar flow

cabinet and the three separate batches were then mixed thoroughly.

#### Fungicides

The following seven fungicides were used in the study:

- ~ Mepronil, 75% a.i. wettable powder, Basitak®, Schering
- ~ Hexaconazole, 5% a.i. suspension concentrate, Anvil®, ICI
- ~ Flusilazole, 20% a.i. dispersible granules, Nustar®, Du Pont
- ~ Fenpiclonil, 40% a.i. flowable suspension, Beret®, Ciba-Geigy
- ~ Prochloraz, 45% a.i. emulsifiable concentrate, Sportak® Schering
- ~ Pencycuron, 25% a.i. flowable suspension, Monceren®, Bayer
- ~ Propiconazole, 25% emulsifiable concentrate, Tilt®, Ciba-Geigy

For the pot experiments and the micro-plot experiment, clay granules (Attapulgit, 100% absorbent granules, Mallina Holdings Ltd, Perth) were coated by thoroughly mixing batches of granules with an appropriate quantity of fungicide in a ball mill. The concentration of active ingredient of fungicide applied to the experiments in this study represented field rates (on 50 kg granules per hectare) of 50, 100 or 200 g  $\text{ha}^{-1}$ . The determination of field rate equivalents has been explained elsewhere (Cotterill *et al.* 1989).

#### Agar plate experiment

To test the activity of fungicides on growth of Ggt and *R. solani* *in vitro*, batches of 20% PDA were amended with 0, 50, 100 or 200 g a.i.  $\text{ha}^{-1}$  equivalents of each fungicide and 15 ml of each batch were poured into Petri dishes. For each isolate of Ggt and *R. solani*, a 6 mm diameter plug from a growing colony was placed in the centre of each plate. There were eight plates of unamended agar and four of each fungicide concentration for each isolate. Plates were randomly arranged, maintained at 25°C and colony diameters were measured after 10 and seven days for Ggt and *R. solani*, respectively. Data for the two pathogens were analysed separately using a three-way analysis of variance.

#### Pot experiment 1

The ability of the fungicides to reduce take-all and rhizoctonia root rot of wheat was examined in this glasshouse experiment. A calcareous sandy loam from Walpeup, Victoria (Chambers 1970) was moistened and amended with 20 ml  $\text{L}^{-1}$  inoculum of Ggt or *R. solani*. Clay granules were added, either unamended (nil control) or amended with each of the fungicides described in the agar plate experiment, to give concentrations equivalent to 100 or 200 g a.i.  $\text{ha}^{-1}$ . One litre of soil mix was dispensed into each of six pots for the nil control and three pots for each concentration of fungicide. Five wheat seeds

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**Table 1. Effect of seven fungicides\* on the colony diameters (cm) of three isolates of *Gaeumannomyces graminis* var. *tritici* and three isolates of *Rhizoctonia solani* after 10 and 7 days growth, respectively, on 20% strength PDA.**

Fungicide treatments	<i>Ggt</i> isolates				<i>R. solani</i> isolates			
	84.8	Mr 9.5	Mr 9.6	Mean	194	204	447	Mean
Nil	6.91	8.08	4.06	6.35	8.09	8.18	4.53	6.93
Mepronil	7.40	8.01	4.97	6.79	6.40	6.33	3.68	5.47
Hexaconazole	1.70	2.78	1.59	2.03	3.83	4.03	2.78	3.54
Flusilazole	3.67	4.53	3.14	3.78	5.97	5.85	3.68	5.17
Fenpiclonil	6.85	7.72	4.58	6.38	5.84	5.50	2.65	4.66
Prochloraz	3.10	3.20	1.63	2.64	7.08	7.86	4.32	6.42
Pencycuron	6.28	8.08	4.14	6.17	6.84	7.44	5.03	6.44
Propiconazole	5.02	5.57	3.51	4.70	5.70	5.90	4.16	5.25
**LSD	P = 0.05	0.58 <sup>A</sup> , 0.53 <sup>B</sup>		0.31	0.69 <sup>A</sup> , 0.63 <sup>B</sup>		0.36	
	P = 0.001	0.99, 0.91		0.52	1.18, 1.08		0.62	

\* Results are means of 50, 100 and 200 g a.i. ha<sup>-1</sup> equivalent concentrations.

\*\* Least significant differences for comparisons between isolate mean values within nil control treatment<sup>A</sup> and between nil control and fungicide treatment values within columns<sup>B</sup> (individual isolates).

**Table 2. Effect of seven fungicides on the severity of take-all and rhizoctonia root rot and the growth of wheat plants in pot experiment 1.**

Fungicide treatment	Take-all				Rhizoctonia root rot			
	Severity (0-5)	Total dry shoot (mg)	Total dry root wt. (mg)	Height (cm)	Severity (0-5)	Total dry shoot (mg)	Total dry root wt. (mg)	Height (cm)
Nil	3.05	180	140	12.6	3.49	70	50	9.4
Mepronil	2.27	250	220	15.6	2.52	100	140	14.4
Hexaconazole	2.50	250	200	12.9	1.27	130	310	16.8
Flusilazole	2.76	270	200	14.7	1.70	60	140	17.0
Fenpiclonil	3.07	170	120	13.0	2.23	130	160	14.8
Prochloraz	2.68	220	170	14.4	2.53	80	160	17.8
Pencycuron	2.56	260	200	16.0	2.03	60	150	15.6
Propiconazole	2.77	230	200	13.0	1.44	150	250	17.9
*LSD P = 0.05	0.69	55	65	1.7	0.68	95	107	4.0

\* Least significant differences for comparisons between nil control and fungicide treatments only.

(cv. Condor) were sown at a depth of 3 cm, pots were arranged in randomized complete blocks and plants were grown at a temperature range of 10-25°C and watered regularly. After eight weeks, plants were harvested and roots were assessed for incidence (percent number of plants infected) and severity of either take-all or rhizoctonia root rot. Severity was measured on 0-5 scales described elsewhere; for take-all (Cotterill *et al.* 1992) and for rhizoctonia root rot (Cotterill *et al.* 1989), where 0 = no disease and 5 = severe disease. Plant heights, shoot and root dry weights/pot were measured. A two-way analysis of variance was performed on take-all and rhizoctonia root rot data separately. Linear regression analyses were carried out to determine the relationship between measures of disease and plant growth, and also between colony diameters of the pathogens on agar and severity of the respective diseases in this experiment.

#### Pot experiment 2

This experiment was conducted to determine the effect of the fungicides on the growth of uninfected plants. The calcareous sandy loam described in pot experiment 1 was pasteurised to kill pathogens by moistening, then steam-treating at 60°C for 30 minutes. The experiment was conducted as for pot experiment 1, except that soil was not amended with inoculum. A 50 g ha<sup>-1</sup> concentration was included for each fungicide in addition to 100 and 200 g ha<sup>-1</sup> concentrations. There were eight replicate pots for the nil control and four for each concentration of each fungicide. A two-way analysis of variance was performed on the data.

#### Micro-plot experiment

Plots of the calcareous sandy loam at Horsham were amended with 1.6 mL inoculum of *Ggt* or *R. solani* per litre of soil to a depth of 5 cm (8000 propagules m<sup>-2</sup>) three weeks prior to sowing. On 11 July 1990, 90 cm slits, 3 cm deep were opened in

the soil and 30 wheat seeds (cv. Condor - 60 kg ha<sup>-1</sup>), 1.3 g of double superphosphate (80 kg ha<sup>-1</sup>) and 0.8 g of clay granules (50 kg ha<sup>-1</sup>), either unamended or amended with hexaconazole, flusilazole, prochloraz or propiconazole (200 g ha<sup>-1</sup>), placed within them, then covered over with soil. For each disease there were two untreated and four fungicide-treated (one per fungicide) single drill row plots randomly arranged in five 1 m<sup>2</sup> blocks. Plots were rainfed and the plants in half of each plot were sampled after eight week's growth. Roots were washed free of soil and assessed for take-all and rhizoctonia root rot as described in pot experiment 1. Plant heights were also measured. Plants in the remaining half of each plot were left until harvest when grain weights were measured. An analysis of variance was performed on the data (which were transformed if necessary) and a linear regression analysis was used to determine the relationship between disease and yield.

## Results

### Agar plate experiment

***Gaeumannomyces graminis* var. *tritici*.** After 10 days growth the colony diameter of isolate Mr 9.5 in unamended agar was greater ( $P < 0.01$ ) than isolate 84.8, which in turn was greater ( $P < 0.001$ ) than isolate Mr 9.6 (Table 1). When fungicides reduced growth of *Ggt* they generally did so at all three concentrations, hence results presented in Table 1 are the means of the 50, 100 and 200 g a.i. ha<sup>-1</sup> equivalent concentrations. Hexaconazole, flusilazole and prochloraz reduced growth of all three *Ggt* isolates ( $P < 0.001$ ) as did propiconazole (84.8 and Mr 9.5 [ $P < 0.001$ ], Mr 9.6 [ $P < 0.05$ ]). Pencycuron reduced the growth of isolate 84.8 ( $P < 0.05$ ), but mepronil and fenpiclonil had no effect.

***Rhizoctonia solani*.** Colony diameter of isolate 447 was less ( $P < 0.001$ ) than isolates 194 and 204 which were similar after seven days growth in unamended agar (Table 1). All fungicides were effective ( $P < 0.05$ ) in reducing growth of *R. solani*, although mepronil, hexaconazole, flusilazole, fenpiclonil and propiconazole were more so ( $P < 0.001$ ) than prochloraz or pencycuron ( $P < 0.05$ ). Hexaconazole and fenpiclonil were effective on all isolates at all concentrations, mepronil and flusilazole were not quite as effective, propiconazole was effective at all concentrations for isolates 194 and 204, but not for 447, pencycuron was only effective at 100 and 200 g for 194, and 200 g for 204 and prochloraz only at 100 and 200 g for 194.

### Pot experiment 1

**Take-all.** There were no differences between the 100 or 200 g concentrations of each fungicide for measures of disease and plant growth, so the concentration means

were used to evaluate fungicide effectiveness (Table 2). None of the fungicides reduced the number of plants infected and only mepronil reduced ( $P < 0.05$ ) disease severity. Despite there being a closer ( $P < 0.001$ ) correlation between disease severity and root dry weight than between severity and shoot dry weight ( $P < 0.01$ ) (Table 3), only mepronil increased ( $P < 0.05$ ) root weight, whereas mepronil, hexaconazole, flusilazole and pencycuron increased ( $P < 0.05$ ) shoot weight (Table 2). There was also a significant ( $P < 0.001$ ) relationship between disease severity and height (Table 3), with mepronil, flusilazole, prochloraz and pencycuron all increasing heights (Table 2).

**Rhizoctonia root rot.** None of the fungicides reduced the number of plants infected or increased shoot weight, but all reduced ( $P < 0.05$ ) disease severity and increased ( $P < 0.05$ ) plant height. Hexaconazole, fenpiclonil, prochloraz and propiconazole increased ( $P < 0.05$ ) root weight (Table 2). There were significant correlations between disease severity and shoot weight ( $P < 0.05$ ), root weight ( $P < 0.001$ ) and plant height ( $P < 0.001$ ) (Table 3).

There was no correlation between colony diameter of either *Ggt* or *R. solani* on agar and severity of take-all and rhizoctonia root rot, respectively, in the pot experiment.

#### Pot experiment 2

Fungicides generally had no effect on the growth of plants in the absence of root disease, although prochloraz, at a concentration equivalent to  $100 \text{ g ha}^{-1}$ , reduced ( $P < 0.05$ ) shoot dry weight from 579 to 392 mg (LSD = 159). Plant heights were reduced ( $P < 0.05$ ) by prochloraz at 100 g (from 24.9 to 18.9 cm, LSD = 2.99) and at 200 g by hexaconazole, fenpiclonil and propiconazole (21.5, 21.9 and 19.8 cm, respectively).

#### Micro-plot experiment

**Take-all.** Disease incidence was reduced ( $P < 0.05$ ) by hexaconazole, prochloraz and propiconazole, but disease severity only by prochloraz and propiconazole ( $P < 0.05$ ) (Table 4). None of the fungicides increased plant height despite there being a significant ( $P < 0.001$ ) correlation between height and disease severity ( $y = 20.59 - 2.48x$ ,  $r^2 = 0.31$ ). Only prochloraz and hexaconazole increased ( $P < 0.05$ ) grain yield (Table 4). There were significant correlations between both measures of disease and  $\log_{10}$  (grain yield + 1); for incidence,  $y = 1.210 - 0.007x$ ,  $P < 0.005$ ,  $r^2 = 0.25$  and for severity,  $y = 1.25 - 0.274x$ ,  $P < 0.05$ ,  $r^2 = 0.17$ .

**Rhizoctonia root rot.** Hexaconazole and propiconazole reduced ( $P < 0.05$ ) disease incidence and all four fungicides reduced

**Table 3. Linear regression equations of measures of plant growth on the severity of take-all or rhizoctonia root rot in pot experiment 1.**

Dependent variable	Regression equation	Significance level	$r^2$
<b>Take-all</b>			
Total dry shoot weight*	$y = 0.32 - 0.03x$	$P < 0.01$	0.13
Total dry root weight	$y = 0.35 - 0.06x$	$P < 0.001$	0.38
Plant height	$y = 18.46 - 1.62x$	$P < 0.001$	0.27
<b>Rhizoctonia root rot</b>			
Total dry shoot weight	$y = 0.15 - 0.03x$	$P < 0.05$	0.06
Total dry root weight	$y = 0.34 - 0.08x$	$P < 0.001$	0.41
Plant height	$y = 22.24 - 3.15x$	$P < 0.001$	0.46

\* Weight data in regression analyses were in g, whereas they are expressed as mg in Table 2.

**Table 4. Effect of four fungicides on the incidence and severity of take-all, rhizoctonia root rot and growth of wheat in micro-plot experiment.**

Fungicide treatment	Take-all				Rhizoctonia root rot			
	Incidence (%)	Severity (0-5)	Height (cm)	$\log_{10}$ (grain wt+ 1) (g/plot)	Incidence (%)	Severity (0-5)	Height (cm)	Grain wt. (g/plot)
Nil	92.5	2.32	15.9	0.46	68.2	1.61	17.1	11.18
Hexaconazole	59.0	1.84	17.3	0.92	28.6	1.17	17.9	13.17
Flusilazole	64.8	2.03	13.7	0.75	48.0	1.19	18.2	9.53
Prochloraz	60.2	1.39	18.1	1.00	49.4	1.09	18.7	12.81
Propiconazole	60.4	1.64	14.1	0.65	29.6	1.04	12.7	4.35
* LSD $P = 0.05$	31.6	0.66	2.7	0.42	21.2	0.33	2.4	7.83

\* Least significant differences for comparisons between nil control and fungicide treatments only

( $P < 0.05$ ) disease severity. None of the fungicides had any effect on plant heights or grain yield, with the exception of propiconazole, which decreased ( $P < 0.05$ ) heights (Table 4). There were no correlations between measures of disease and grain yield.

#### Discussion

Although several of the fungicides showed promise in the control of both take-all and rhizoctonia root rot throughout this study, only prochloraz and hexaconazole increased the grain yield of take-all infected plants in outdoor micro-plots and no yield increases were recorded for fungicide-treated plants infected with rhizoctonia root rot. This suggests that, in the case of rhizoctonia root rot, either that the reduction in disease severity from a relatively low 1.61 to 1.19-1.04 was insufficient to elicit a yield response or that all the fungicides tested are probably ineffective as a practical means of controlling this disease under field conditions.

The results of the agar plate experiment, pot experiment 1 and micro-plot experiment highlight the inconsistencies which may occur between different screening techniques. The use of agar plates to measure the effect of a fungicide on the in vitro growth of *Ggt* cannot be related to its effect on take-all in soil, with hexaconazole, flusilazole and prochloraz highly effective on agar but ineffective in plots and

mepronil behaving in the opposite manner. This finding concurs with previous results using different fungicides at similar (Cotterill *et al.* 1992) and much higher rates (Bateman *et al.* 1990). Nevertheless, there was consistency between all screening methods for *R. solani* and rhizoctonia root rot and although there was no correlation between colony diameter of *R. solani* on agar plates and disease severity in the pot experiment, all fungicides reduced growth of *R. solani* in vitro and reduced severity of disease on wheat. Thus in this study the agar plate test of the effectiveness of fungicides on growth of the pathogen *in vitro* provided a good indication of their effectiveness against the severity of disease in pots. However, this technique was unable to differentiate the order of effectiveness of the individual fungicides. In micro-plots, the four fungicides tested reduced either or both incidence and severity of rhizoctonia root rot, but none had any effect on yield. Studies elsewhere (Smiley *et al.* 1990a,b) also indicated the effectiveness of fungicides in controlling the growth of *R. solani* in vitro, but control in the field was generally ineffective and unreliable and none of them consistently improved grain yield.

There was little effect of concentration on the performance of fungicides, in vitro they were generally as effective at 50, as at 100 or 200 g a.i.  $\text{ha}^{-1}$  equivalents and similarly in the pot experiment there were no

differences between 100 and 200 g concentrations for each fungicide. From a commercial standpoint this is important, the lower the rate required to effect disease control, the lower the cost. Additionally, the greater the concentration the more likely a fungicide will retard plant growth, as indicated by the results in pot experiment 2 where hexaconazole, fenpiclonil and propiconazole reduced plant heights at 200 g ha<sup>-1</sup> equivalents. Prochloraz, however, reduced shoot weight and height only at 100 and not at 200 g ha<sup>-1</sup>, an inexplicable result. Overall, the fungicides used in this study were not particularly growth-retarding or growth-stimulating in the absence of disease.

There are perhaps some risks involved in attempting to determine the effectiveness of fungicides on two diseases which may occur together in the field, separately but under the same environmental and soil conditions as in this study. The reason why some of these fungicides may appear more effective against rhizoctonia root rot than against take-all could be related to characteristics of the two diseases. Although similar levels of take-all and rhizoctonia root rot were recorded on untreated plants in pot experiment 1, plant weights were greater in take-all infected plants. As rhizoctonia root rot is considered to be a seedling disease (Samuel and Garrett 1932), assessing the effectiveness of fungicides after only eight weeks plant growth may indicate that, as the results of this study suggest, they are generally more effective against rhizoctonia root rot than against take-all, a disease which in the field generally has a serious effect on plants at a later stage of their development. However, the ability of a fungicide to reduce early disease does not necessarily mean that this will be reflected in improved yields, the fungicide may not persist, enabling the pathogen to re-infect the plant roots at a later stage. If fungicides are able to control take-all very early on, as mepronil, hexaconazole, prochloraz and propiconazole did, then it is possible that effective disease control could be achieved, if the fungicides were persistent.

This study has failed to identify a fungicide which is effective against both take-all and rhizoctonia root rot under field conditions at potentially commercial concentrations. Mepronil, which was not included in the field tests, possibly warrants further testing in this regard, although this study has indicated that the ability of a fungicide to reduce disease in pots is no guarantee that it will be effective in field plots.

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