Preventative control of take-all patch of bentgrass turf using triazole fungicides and *Gaeumannomyces graminis* var. *graminis* following soil fumigation

P.T.W. Wong, Biological and Chemical Research Institute, Rydalmere, New South Wales 2116, Australia.

D.J. Worrad, Australian Turfgrass Research Institute, 68 Victoria Avenue, Concord West, New South Wales 2138, Australia.

Summary

***In vitro* studies showed that the mycelial growth of two isolates of *G. graminis* var. *avenae* was completely inhibited by triadimefon at 100 μg ml^{-1}, by triadimenol at 10 μg ml^{-1} and by propiconazole at 1 μg ml^{-1}. The mycelial growth of three isolates of *G. graminis* var. *graminis*, a fungal antagonist of the pathogen, was completely inhibited by triadimefon at 10 μg ml^{-1} and by triadimenol and propiconazole at 1 μg ml^{-1}.**

In a glasshouse experiment, take-all patch was effectively controlled on bentgrass turf cv. Penncross by 4-weekly applications of triadimefon (15 g a.i. 100 m^{-2}) and propiconazole (15 g a.i. 100 m^{-2}). A single application of the fungicides at seeding was not as effective and disease occurred after 6 weeks. The fungal antagonist, *G. graminis* var. *graminis*, gave a moderate level of control.

In a field experiment, where the soil had been fumigated with methyl bromide and then sown to Browntop bentgrass, applications of triadimenol (15 g a.i. 100 m^{-2}) or propiconazole (15 g a.i. 100 m^{-2}) at 1, 10 and 19 weeks after inoculation with the pathogen controlled the disease, while bitertanol (15 g a.i. 100 m^{-2}) did not. *G. graminis* var *graminis* again achieved moderate suppression of the disease. The field results show that take-all patch may be prevented for a year after soil fumigation by three 9-weekly applications of triadimenol or propiconazole.

Introduction

There are no effective control measures against take-all patch of bentgrass (*Agrostis* L. spp.) turf caused by *Gaeumannomyces graminis* (Sacc.) Arx and Olivier var. *avenae* Turner (Smiley 1983). Cultural methods such as keeping the soil in the acid range (pH 5.0-6.0) by fertilizing with ammonium sulfate and the use of coarser grades of limestone when liming the soil have been recommended (Wong and Baker 1985). Of the chemicals tried, chlordane has suppressed the disease (Gould *et al.* 1966) and has been used in Australia in the past. However, the use of chlordane has now been banned in Australia and other countries.

In the United Kingdom, biological control of the disease by the careful management of indigenous populations of *Phialophora graminicola* (Deacon) Walker in golf greens has been proposed by Deacon (1973). This entailed keeping the soil slightly acid and by judiciously applying fungicides. Another fungal antagonist, *Gaeumannomyces graminis* var. *graminis*, has been shown to control the disease in pot experiments (Wong and Siviour 1979) but has not been tested in the field.

Recently, Chastagner and Staley (1986) in the United States of America have reported that fenarimol, triadimefon and propiconazole at a rate of 30 g a.i. 100 m^{-2} or higher were effective in suppressing disease symptoms of take-all patch when applied in winter but failed when applied in spring. The reasons for the failure are unclear. Other triazole fungicides such as triadimenol and flutriafol have also been used successfully to control wheat take-all (*G. graminis* var. *tritici* Walker) in the field (Ballinger and Kollmorgen 1988). This suggests that the triazole group of fungicides may be effective in preventing take-all disease in turf if applied at seeding.

In Australia, take-all patch of bentgrass is usually only a problem in the first or second year following the fumigation of greens using methyl bromide. As such, this study was undertaken to attempt to prevent disease occurring in bentgrass turf following soil fumigation by the use of triazole fungicides and the biological antagonist *G. graminis* var. *graminis*.

Materials and methods

In vitro *studies*

The fungal isolates used in the experiments are listed in Table 1. Molten quarter-strength potato dextrose agar (¼ PDA) at 45°C was amended with 0 (control), 1, 10 and 100 μg of triadimenol, triadimefon or propiconazole per ml of medium. Fifteen ml of each medium were dispensed into sterile plastic petri dishes. The fungi were grown on ¼ PDA at 25 ± 1°C for 4 days and 5 mm plugs were taken at the edge of the colonies and placed at the centre of the fungicide-amended plates. There were three replicates per treatment. The plates were incubated at 25 ± 1°C and the radii of the colonies were measured after 5 and 7 days.

Glasshouse experiment

A sandy loam (pH 7.0) was autoclaved at 121°C for 1 h on 2 days. Plastic pots, ten cm in diameter, were filled to 3 cm from the top with the sterilized soil. Ground oat inoculum of the pathogen (Wong and Siviour 1979) was sprinkled on the soil surface at a rate of 0.1 g per pot and covered with a further 1 cm depth of sterile soil. Bentgrass seed (cv. Penncross) was sown and thinly covered with sterilized soil.

The treatments were: 1, untreated control; 2, inoculated with pathogen; 3, inoculated + *G. graminis* var. *graminis*; 4, inoculated + triadimefon (once); 5, inoculated + triadimefon (every 4 weeks); 6, inoculated + propiconazole (once); 7, inoculated + propiconazole (every 4 weeks).

The antagonist, *G. graminis* var. *graminis* (DAR 24167) was mixed with the seed at sowing as ground oat inoculum (0.1 g per pot). The fungicides were added after sowing at the rate of 15 g a.i. 100 m^{-2} as an aqueous drench. Some treatments had 4-weekly applications of fungicides. There were four replicates per treatment and the pots were randomized in blocks in a glasshouse kept at 5°C for 12 h and 15°C for 12 h. The pots were watered daily to field capacity and fertilised every second week with Hoagland's solution (Hoagland and Arnon 1939).

After 6 weeks when take-all patch symptoms were obvious, the growth of grass was rated for disease severity on the following scale: 0, no disease; 1, up to 25% of pot area diseased; 2, from 26 to 50% of pot area diseased; 3, from 51 to 75% of pot area diseased; 4, from 75 to 100% of pot area diseased. After 10 weeks, the pots were again assessed for disease.

Field experiment

This experiment was on a site at the Australian Turfgrass Research Institute. The site

Table 1. Fungi used in experiments

Fungus	Isolate	Host	Locality
Gaeumannomyces graminis var. *avenae*	DAR 23143	*Agrostis palustris* Huds.	Concord, N.S.W.
"	DAR 37725	*Agrostis* sp.	Tumut, N.S.W.
Gaeumannomyces graminis var. *graminis*	DAR 24167	*Stipa aristiglumis* F. Muell.	Warialda, N.S.W.
"	DAR 33669	*Aristida* sp.	Tamworth, N.S.W.
"	DAR 33671	*Paspalidium* sp.	Walcha, N.S.W.

was fumigated with methyl bromide (0.65 kg 100 m^{-2}) and left uncovered for 2 weeks. Plots (1 m x 1 m) received the following treatments in a randomized complete block design with four replicates: 1, untreated; 2, inoculated with the pathogen; 3, inoculated + *G. graminis* var. *graminis*; 4, inoculated + triadimenol (15 g a.i. 100 m^{-2}); 5, inoculated + propiconazole (15 ml a.i. 100 m^{-2}); 6, inoculated + bitertanol (15 g a.i. 100 m^{-2}):

Ground oat inoculum (20 g per plot) of *G. graminis* var. *graminis* (DAR 24167) was sprinkled over the plot area and lightly raked in. Two days later the whole trial site was sown with Browntop bentgrass (*Agrostis capillaris* L.) at rate of 0.91 kg 100 m^{-2}. Four weeks after seeding, the plots were inoculated with 5 g of colonized whole millet inoculum (Simon and Rovira 1985) of *G. graminis* var. *avenae* (DAR 37725), by cutting a 5 cm plug (3 cm deep) in five spots per plot, dropping the inoculum in each spot and covering the hole with the turf plug. The fungicides were applied with a hand-held boom spray one week after inoculating with the pathogen.

The trial site was watered and maintained as a golf green with the exception that the soil was kept at around pH 7.0 by liming and that nitrogen was applied in the form of calcium nitrate (2 kg 100 m^{-2}) every eight weeks. Seventeen weeks after seeding, when disease symptoms first appeared, the plots were assessed for disease by counting the number of inoculated spots which showed disease. After a further four weeks, when large patches of dying grass were obvious, the number of patches and the diameter of the patches were measured.

Results

In Vitro *Studies*

The growth of two isolates of *G. graminis* var. *avenae* was completely inhibited by triadimefon at 100 $\mu g\ ml^{-1}$, by triadimenol at 10 g ml^{-1} and by propiconazole at 1 $\mu g\ ml^{-1}$ (Table 2). The mycelial growth of *G. graminis* var. *graminis* was completely inhibited by triadimefon at 10 $\mu g\ ml^{-1}$ and by triadimenol and propiconazole at 1 $\mu g\ ml^{-1}$.

Glasshouse experiment

After 6 weeks, severe disease was observed in the pots inoculated with the pathogen only (Table 3). Significantly less disease ($P \leq 0.05$) was present in pots inoculated with the pathogen and the fungal antagonist and pots drenched with triadimefon and propiconazole. One application of propiconazole gave a significantly ($P \leq 0.05$) higher level of control compared to one application of triadimefon.

The second disease assessment at 10 weeks showed similar results except that the propiconazole treatments completely suppressed the disease.

Table 2. Effect of triazole fungicides on the growth of *Gaeumannomyces graminis* var. *avenae* and var. *graminis* on agar.

Fungus	Radial growth (mm) after 7 days at various concentrations of chemicals (g ml^{-1})									
	Control	Triadimefon			Triadimenol			Propiconazole		
		1	10	100	1	10	100	1	10	100
G. graminis var. *avenae*										
DAR 23143	40.7±0.6*	27.7±1.2	40±0	0	10±0	0	0	0	0	0
DAR 37725	34.7±0.6	31.7±0.6	10.0±0	0	4.7±0.6	0	0	0	0	0
G. graminis var. *graminis*										
DAR 24167	39.7±0.6	20±0	0	0	0	0	0	0	0	0
DAR 33669	44.7±1.2	20±0	0	0	0	0	0	0	0	0
DAR 33671	44.3±1.2	4.7±0.6	0	0	0	0	0	0	0	0

* Standard deviation of mean of three replicates.

Table 3. Effect of triazole fungicides and *G. graminis* var. *graminis* on take-all patch of bentgrass in a glasshouse experiment.

Treatments	Disease Index*	
	A**	B**
1 Uninoculated	0.0	0.0
2 Inoculated with pathogen	4.0	3.5
3 Inoculated + *G. graminis* var. *graminis*	1.3	1.0
4 Inoculated + triadimefon (once)	1.8	1.5
5 Inoculated + triadimefon (every 4 weeks)	0.3	0.3
6 Inoculated + propiconazole (once)	0.3	0.0
7 Inoculated + propiconazole (every 4 weeks)	0.0	0.0
L.s.d. (P = 0.05).	0.8	0.9

* Refer to text.
** A = assessed after 6 weeks. B = assessed after 10 weeks.

Table 4. Effect of triazole fungicides and biological antagonist on take-all patch of bentgrass in a field experiment.

Treatment	Mean number of diseased patches per plot		Total diameter of disease patches (cm)
	A*	B*	B*
1. Uninoculated	0.0	0.5	8.5
2. Inoculated with pathogen	3.3	4.0	76.7
3. Inoculated + *G. graminis* var. *graminis*	0.5	2.2	33.5
4. Inoculated + triadimenol	0.0	0.0	0.0
5. Inoculated + propiconazole	0.0	0.0	0.0
6. Inoculated + bitertanol	1.3	5.2	74.2
L.s.d. (P = 0.05)	2.5	2.4	39.5

* A = Disease assessed 13 weeks after inoculation with the pathogen.
* B = Disease assessed 17 weeks after inoculation with the pathogen.

Field experiment

Thirteen weeks after inoculating the pathogen, small disease patches were obvious at the points of inoculation on some of the plots. The mean number of disease patches per plot was determined (Table 4). Plots sprayed with triadimenol and propiconazole were free of disease. Those sprayed with bitertanol or inoculated with the fungal antagonist had significantly ($P \leq 0.05$) fewer disease patches than the plots inoculated with the pathogen.

After a further 4 weeks (when large disease patches were obvious on the control plots), a second disease assessment was made. Plots sprayed with triadimenol and propiconazole were again free of disease. The uninoculated plots showed a low level of disease. Plots inoculated with the fungal antagonist

showed moderate infection which was not significantly different to the plots inoculated with the pathogen alone. However, the total diameter of the disease patches in the plots inoculated with the fungal antagonist was significantly less ($P \leq 0.05$) than that of the plots inoculated with the pathogen alone. Plots sprayed with bitertanol were as severely diseased as those inoculated with the pathogen alone.

A year after sowing the bentgrass, irregular disease patches were still discernible in many plots where the pathogen had been inoculated. However, the symptoms were less obvious and the patches were highly irregular in outline. No disease assessment was made but it was observed that there was an absence of disease patches in plots where triadimenol or propiconazole had been applied three times.

Discussion

Following the fumigation of soil with methyl bromide, newly-sown bentgrass turf was protected for at least a year from take-all patch by 3 applications of triadimenol (15 g a.i. 100 m^{-2}) or propiconazole (15 g a.i. 100 m^{-2}). The fungicides were first applied 1 week after inoculating with the pathogen when the turf was 4 weeks old. Both fungicides caused phytotoxic effects with the grass becoming yellow and growth retarded compared to the unsprayed plots. However, the grass recovered after 6 weeks and a second application of the fungicides did not cause visible phytotoxic effects.

Bitertanol (15 g a.i. 100 m^{-2}) did not provide any control after 17 weeks. *G. graminis* var *graminis* gave a moderate level of control in the field but is probably insufficient to be of practical value.

A year after sowing the bentgrass, it was observed that take-all patch disease was still absent from plots which had been sprayed three times with either triadimenol or propiconazole. It is anticipated that a further two or three sprays in the second year would provide sufficient time for natural biological suppression to build up so that further sprays would not be necessary. However, these sprays would be incompatible with the use of the fungal antagonist since *G. graminis* var. *graminis* is highly susceptible to the fungicides. It is not known at this stage whether these fungicides have any curative effect once the disease has occurred. Experiments are continuing to study this.

Acknowledgment

We thank W.A. Flick & Co. for fumigating the field site prior to the commencement of the field experiment; Mr H.C. Kirton for statistical advice and assistance; Mr L.A. Tesoriero and Mr G. Hollihan for technical and field assistance.

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