EFFECTS OF FUNGICIDES APPLIED AT ANTHESIS ON FUSARIUM HEAD BLIGHT AND MYCOTOXINS IN WHEAT

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ABSTRACT

Fusarium head blight (FHB) of wheat can cause yield losses of 30-70%. More importantly, affected grain may be less palatable to stock than healthy grain and may contain mycotoxins. A field trial in 2000/01 tested several fungicides and fungicide combinations for FHB control. FHB incidence was 9.1% in untreated plots. The greatest reduction of FHB incidence, grain *Fusarium* and mycotoxins, was achieved with triazoles, a benzimidazole, or a combination of these. Strobilurin fungicides reduced FHB, although less effectively than the triazoles and carbendazim, but *Fusarium* incidence was greater in grain harvested from strobilurin-treated plots than in grain from untreated plots. Combining strobilurin fungicides with fungicides from other groups did not further reduce *Fusarium* levels in harvested grain. Nine *Fusarium* species and *Microdochium nivale* were isolated from grain harvested from the trial. *Fusarium graminearum* and *F. avenaceum* were most commonly isolated, with *F. crookwellense* and *F. culmorum* also common.

Keywords: head scab, *Fusarium graminearum*, *F. avenaceum*, triazole, strobilurin.

INTRODUCTION

Fusarium head blight (FHB) of wheat, also known as head scab, is most easily recognised on immature heads when one or more spikelets in each head appear prematurely bleached. Sometimes large areas of heads may be affected, and when severe, pink or orange spore masses can be seen on diseased spikelets. FHB can cause yield losses of 30-70% where conditions favour the disease. Feed grain from affected crops may be less palatable to stock than healthy grain and may contain mycotoxins (Bai & Shaner 1994).

There are two stages of infection of wheat ears by *Fusarium*. Wheat is most susceptible to primary infection during flowering when florets are infected, especially during wet conditions (Bai & Shaner 1994). Secondary spread between florets and spikelets occurs at later stages of crop development. Recent overseas research suggests that tebuconazole may provide a degree of control of FHB, but only if applied at close to mid-flowering (Suty et al. 1996). Control of secondary disease spread within ears has not yet proved possible. Trials in New Zealand in the 1998/99 and 1999/2000 growing seasons showed that applications of fungicides at various crop growth stages around flowering reduced the incidence of FHB in spring wheat (Crome et al. 2001). This paper describes a further trial in the 2000/01 growing season which examined a wider range of fungicides and fungicide combinations applied during flowering, the optimal time of application.

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METHODS

The trial was sown on 26 September 2000 and was located in the Kairanga district of Manawatu where FHB is common. The trial used the cultivar Otane, which is highly susceptible to FHB. Plot size was 1.2 m x 5 m, in a randomised complete block design with four replicates. The blocks were arranged sequentially in a single row of plots. Two nil fungicide plots were included in each replicate. Treatments are listed in Table 1.

TABLE 1: Fungicides applied to wheat for *Fusarium* head blight control.

Treatment	Active ingredient	Application rate (g ai/ha)
Nil	-	-
Folicur 430SC	tebuconazole	189
Opus	epoxiconazole	125
Bavistin DF	carbendazim	250
Amistar	azoxystrobin	125
Folicur 430SC	tebuconazole	94.5 (half rate)
Folicur 430SC + Amistar	tebuconazole + azoxystrobin	189 + 125
Bavistin DF + Amistar	carbendazim + azoxystrobin	250 + 125
Twist	trifloxystrobin	125
BAS512	pyraclostrobin + epoxiconazole	rate confidential
Bavistin DF + Folicur 430SC	carbendazim + tebuconazole	250 + 189

For treated plots, each plot was sprayed at mid-anthesis (12 December 2000) with a half rate of fungicide in one direction, followed by a half rate in the opposite direction, to ensure even coverage of fungicide on ears. Plots were sprayed with a Hardi 4100-12 fan nozzle on a hand-held boom with a knapsack sprayer, applying 300 litres/ha at 300 kPa.

The percentage of ears infected with FHB was assessed on 9 January 2001, at the milky ripe stage of crop development, by examining approximately 500 ears per plot for FHB symptoms. The trial was harvested on 2 February 2001. Plot yields (adjusted to 14% moisture) and thousand grain weights were measured.

One hundred seeds per plot (sampled from the harvested grain) were surface sterilised in 1% sodium hypochlorite for 3 min, and plated on to Potato Dextrose Agar at 10 seeds per plate. Plates were incubated for 4 to 8 days at room temperature under conditions of alternating light and dark (12 h photoperiod) and then numbers of *Fusarium* colonies were counted. Species identification was carried out for fungal colonies on PDA and on Carnation Leaf Agar where required. Grain samples were analysed for the presence of mycotoxins as described by Cromey et al. (2001).

All data were statistically analysed with analysis of variance. For the mycotoxin data, treatments with a value of 0 for all replicates were excluded from the ANOVA; these excluded treatments were subsequently compared using results computed as part of the ANOVA.

RESULTS

Fusarium head blight (FHB) incidence was 9.1% in untreated plots at the milky ripe stage of crop development (Table 2). All fungicide treatments reduced ($P < 0.05$) FHB incidence compared with the nil fungicide treatment. Fungicides could be grouped into two categories. One group (comprising carbendazim, tebuconazole, BAS512 and carbendazim + tebuconazole) reduced FHB incidence by 75% or more. The other group (comprising azoxystrobin, trifloxystrobin, azoxystrobin + carbendazim and azoxystrobin + tebuconazole) reduced levels to approximately 50% of that of the nil fungicide treatment. No foliar diseases were recorded in the trial.

Yields were not affected ($P > 0.05$) by fungicide treatment (Table 2). Yields varied between 5.1 and 5.5 t/ha, with the nil fungicide treatment being in the middle of the

range. Grain weights were 6% higher ($P<0.05$) following treatment with epoxiconazole, BAS512, or carbendazim + tebuconazole than if left untreated. Grain weights from the nil fungicide plots were at the bottom of the range in the trial.

Fusarium incidence on harvested grain from nil fungicide plots was 6.4% and there were statistically significant differences recorded between treatments (Table 2). There was a trend for the fungicides azoxystrobin, trifloxystrobin and BAS512 to result in higher infection levels than the nil fungicide treatment, while infection levels tended to be lower than the nil fungicide treatment following treatment with tebuconazole, epoxiconazole, carbendazim, and carbendazim + tebuconazole. The fungicide mixtures azoxystrobin + carbendazim and azoxystrobin + tebuconazole tended to give intermediate *Fusarium* incidence on grain between their respective fungicides applied alone.

TABLE 2: Mean *Fusarium* head blight (FHB) incidence, yields, grain weights, *Fusarium* incidence and mycotoxins nivalenol (NIV) and deoxynivalenol (DON) in grain in plots that were either untreated or treated with fungicides at mid anthesis.

Treatment	Ears with FHB (%)	Yield (t/ha)	Grain weight (mg) (%)	Grains with <i>Fusarium</i>	NIV (mg/kg)	DON (mg/kg)	NIV + DON (mg/kg)
Nil fungicide	9.1	5.3	51	6.4	0.17	0.15	0.32
Tebuconazole	1.5	5.3	52	3.8	0.05	0.01	0.06
Epoxiconazole	2.3	5.2	54	5.8	0.07	0.03	0.10
Carbendazim	1.3	5.1	53	4.8	0	0	0
Azoxystrobin	4.8	5.3	51	10.3	0.12	0.18	0.30
Tebuconazole (half rate)	2.3	5.2	53	4.8	0	0.03	0.03
Tebuconazole + azoxystrobin	4.0	5.3	53	8.0	0.03	0	0.03
Carbendazim + azoxystrobin	5.5	5.4	52	6.8	0.11	0	0.11
Trifloxystrobin	5.0	5.1	52	9.5	0.05	0.11	0.16
BAS512	2.0	5.2	54	9.3	0.03	0.15	0.18
Carbendazim + tebuconazole	1.5	5.5	54	3.5	0	0.01	0.01
LSD ($P<0.05$) (df=34) to compare:							
Nil vs any treatment	2.9	0.4	2.5	3.6	0.11	0.06	0.08
Any two treatments	3.4	0.4	2.9	4.2	0.13	0.09	0.11
Nil vs 0					0.06	0.06	0.08
Any treatment vs 0					0.09	0.09	0.11

Levels of the mycotoxins nivalenol (NIV) and deoxynivalenol (DON) differed between treatments (Table 2). DON levels ranged between 0.00 to 0.18 mg/kg and NIV levels between 0.00 to 0.17 mg/kg. The nil-fungicide and azoxystrobin treatments gave relatively high levels of mycotoxins, whereas levels were lower in plots treated with tebuconazole and carbendazim. Intermediate levels were recorded following treatment with trifloxystrobin or BAS512.

FHB incidence in the nil fungicide plots was greater ($P<0.05$) than for fungicides in general (Table 3). The strobilurin group had higher ($P<0.05$) FHB incidence than the other groups (triazoles, carbendazim or BAS512). There was some evidence that FHB incidence was higher in the mixtures containing azoxystrobin than in the carbendazim + tebuconazole mixture.

TABLE 3: Mean *Fusarium* head blight (FHB), *Fusarium* incidence and mycotoxins (NIV + DON) in grain from plots that were either untreated or treated at mid anthesis with triazole (tebuconazole or epoxiconazole) or strobilurin (azoxystrobin or trifloxystrobin) or other fungicides or mixtures.

Treatment	Ears with FHB (%)	Grains with <i>Fusarium</i> (%)	NIV + DON (mg/kg)
Nil fungicide	9.1	6.4	0.33
Triazole	2.0	4.8	0.09
Strobilurin	4.9	9.9	0.23
Carbendazim	1.3	4.8	0.00
Strobilurin + other	3.8	8.0	0.11
Carbendazim + triazole	1.5	3.5	0.01

There were statistically significant differences between types of fungicides in incidence of *Fusarium* and levels of mycotoxins in grain (Table 3). *Fusarium* incidence and mycotoxin levels were lower after treatment with carbendazim and the triazoles than for the strobilurins. There was evidence of a difference between fungicide mixtures, with the carbendazim + tebuconazole mixture giving lower *Fusarium* incidence and mycotoxin levels than the two strobilurin mixtures.

Nine *Fusarium* species, and *Microdochium nivale* (= *F. nivale*), were isolated from grain harvested from the trial (Table 4). *Fusarium graminearum* and *F. avenaceum* were the species most commonly isolated (71% of isolations), while *Fusarium crookwellense* and *F. culmorum* were also common. *Fusarium acuminatum*, *F. equiseti*, *F. poae* and *F. sambucinum* were isolated infrequently. *Fusarium heterosporum* and *Microdochium nivale* were isolated only rarely.

TABLE 4: Relative occurrence of each *Fusarium* species on wheat grains in plots that were untreated with fungicides in the 2000/01 season.

	Percentage of isolates
<i>Fusarium graminearum</i>	39
<i>F. avenaceum</i>	32
<i>F. crookwellense</i>	13
<i>F. culmorum</i>	7
<i>F. acuminatum</i>	3
<i>F. equiseti</i>	3
<i>F. poae</i>	3
<i>F. sambucinum</i>	2
<i>F. heterosporum</i>	<1
<i>Microdochium nivale</i>	<1

DISCUSSION

Severe FHB was not encountered in disease surveys of wheat crops in the 2000/01 growing season (M.G. Cromey, unpubl. data). Rain fell on 1 day during the flowering period of the trial, and then not again until almost 2 weeks after flowering, when 60 mm rain was recorded over a 2 day period. The generally dry weather during flowering was probably the major factor resulting in FHB incidence in the trial and *Fusarium* incidence and mycotoxin levels in harvested grain being substantially lower than in trials in the same district in the previous two growing seasons (Cromey et al. 2001). *Fusarium*

incidence in grain harvested from untreated plots in 2001 was 6%, compared with 17% and 47% in 1999 and 2000 respectively. Mycotoxin levels were 0.3 mg/kg compared with 2.6 and 2.3 mg/kg in the previous trials. All three trials followed maize, and maize residues can provide high levels of *Fusarium* inoculum for following wheat crops (Bai & Shaner 1994). It is likely that rainfall differences between years during flowering and, to a lesser extent during grain fill, are responsible for the differences in FHB and grain *Fusarium* incidence. Sayer & Lauren (1991) recorded wide variation in infection levels between crops in a three-year survey.

The fungicides differed in their effect on FHB incidence, grain *Fusarium* and mycotoxin levels, but the greatest differences were between fungicide groups. Greatest reduction of FHB, grain *Fusarium* and mycotoxin levels was achieved with triazoles or carbendazim, or a combination of these. The triazole fungicides tebuconazole and epoxiconazole, along with the benzimidazole fungicide carbendazim provided a useful level of FHB control, reducing FHB incidence by 75% to 86%, *Fusarium* incidence by 10% to 45%, and mycotoxin levels by 42% to 100%. Similar reductions were recorded following application of tebuconazole or carbendazim (there was no epoxiconazole treatment) in the 1999/2000 trial (Cromey et al. 2001). The combination of tebuconazole with carbendazim did not improve control of FHB compared with the fungicides individually under the low FHB pressure experienced. The combination treatment may, however, provide relatively better FHB control where inoculum and environmental conditions are more favourable for the disease.

While the strobilurin fungicides azoxystrobin and trifloxystrobin reduced FHB by 45% to 47%, this was only a little over half the efficacy achieved with the triazole fungicides and carbendazim. Similarly, azoxystrobin provided a lesser FHB control than tebuconazole in the 1999/2000 trial (Cromey et al. 2001). The partial control of FHB by the strobilurin fungicides did not translate into lower levels of *Fusarium* in grain. *Fusarium* incidence was 48% and 61% higher in grain harvested from plots treated with trifloxystrobin and azoxystrobin, respectively, than in grain from untreated plots. A similar increase was recorded in the 1999/2000 trial (Cromey et al. 2001). It is likely that strobilurins slow the development of FHB, but do not prevent infection at flowering. The increase in *Fusarium* in grain may be due to the death of natural biological control agents by strobilurin treatments. Combining azoxystrobin with tebuconazole or carbendazim resulted in similar FHB levels to azoxystrobin alone, but *Fusarium* incidence was intermediate between the fungicides individually. As in a previous trial (Cromey et al. 2001), azoxystrobin did not reduce mycotoxin levels in harvested grain, although in the present trial application of the strobilurin fungicide trifloxystrobin resulted in a 50% reduction. Combinations of strobilurins with other fungicide groups did not have a consistent effect on mycotoxin levels, and further work is necessary.

While *F. graminearum* was the most common *Fusarium* species in grain in this trial and also in the two previous years, proportions of species varied between years. *Fusarium graminearum* represented 39% of isolates in 2000/01 compared with 89% in 1999/2000 (Cromey et al. 2001). *Fusarium avenaceum* was not detected in 1998/99, represented only 3% of isolates in 1999/2000 (Cromey et al. 2001), but was almost as common as *F. graminearum* in 2000/01, at 32% of isolations. *Fusarium crookwellense* was common in 1998/99 and 2000/01 (13% of isolations), but represented only 2% of isolations in 1999/2000. While all the species recorded have been found to be associated with FHB in other studies (Bai & Shaner 1994), they vary in their level and type of toxigenicity (Lauren et al. 1992). Inoculation experiments have shown that different *Fusarium* species differ in humidity requirements for ear infection (Jennings & Turner 1996). The species range, therefore, is likely to differ in particular localities and in different seasonal environments.

Application technique may be important in fungicide efficacy against FHB. Using normal spraying machinery, only one face of ears is sprayed, and only partial redistribution of active ingredient occurs within ears (Suty et al. 1996). In our trial, fungicides were applied in two directions to ensure even coverage. Double fan nozzles, which spray in two opposing directions, may also improve efficacy (Suty et al. 1996).

Fungicide mixtures which include the strobilurins tested in our trial, are likely to provide inferior control of FHB and risk increasing *Fusarium* and mycotoxin levels on harvested grain. It is important, therefore, to consider the risk factors for FHB before making the final choice of late-season fungicide applications. Most New Zealand wheat crops have a low risk of serious levels of FHB and associated *Fusarium* and mycotoxins in harvested grains. Important factors are location (North Island, and to a lesser extent South Canterbury crops are most often affected), cultivar susceptibility, previous crop, and rainfall during flowering and (to a lesser extent) grain fill. Of these factors, rainfall is the least predictable.

FHB of wheat is usually most severe where wheat follows maize in the rotation (Bai & Shaner 1996). The disease can be minimised in such situations with the use of partially resistant cultivars combined with the more effective fungicides applied during flowering. Where wheat does not follow maize, fungicide control of FHB is likely to be necessary only in highly susceptible cultivars. Further work is needed on the effect of spray equipment on the efficacy of fungicides.

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