



PROJECT REPORT No. 285

EFFECTS OF AZOXYSTROBIN ON WHEAT TAKE-ALL

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by

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ABSTRACT

Severity of take-all (*Gaeumannomyces graminis* var. *tritici*) on the roots of wheat was significantly decreased by foliar sprays of azoxystrobin in four of the six field experiments in which they were tested. Decreases in plants with moderate or severe symptoms, compared with untreated, ranged from *c* 20% to more than 80%. These effects on take-all were mostly associated with sprays at the full (250 g ai/ha) rate applied at relatively early growth stages (i.e. GS 31-32; beginning of stem extension) either alone or in combination with a later spray.

The effects of azoxystrobin on take-all are, however, somewhat variable. Its current value as a tool in the practical management of the disease may, therefore, best be seen as providing a potential bonus in certain situations i.e. where applications at the rates and times at which it has so far mostly been tested and been shown to be effective against take-all, can be justified by its predicted effects on other diseases.

In one field experiment, a number of related compounds were also tested (all applied twice to the same plots) but there was no evidence that any of those currently available commercially had effects on take-all that were comparable to the effects of azoxystrobin. An experimental compound had similar effects to azoxystrobin in the field experiment but, in contrast to azoxystrobin, had inconsistent effects when applied to the soil in pot experiments.

SUMMARY

Evidence of significant decreases in take-all (*Gaeumannomyces graminis* var. *tritici*) on winter wheat where azoxystrobin had been applied as a foliar spray was first obtained from an experiment at IACR-Rothamsted in 1998. The result was viewed with some caution at the time because occasional effects on take-all of fungicide sprays (or programmes of sprays) have been seen previously but have, typically, proved impossible to repeat. However, further experiments in 1999, including one at Harper Adams University College, showed similar effects of azoxystrobin on take-all suggesting that it was a real effect although the magnitude of the effect differed between experiments. There were also interactions with cultivars that were significant but inconsistent.

Azoxystrobin had previously been shown to be active against *Gaeumannomyces* spp. but its effects on take-all in crops when applied as a foliar spray are difficult to explain from its predicted behaviour in plants and soil. Such effects may, nevertheless, contribute to yield responses in crops at risk from the disease and, if confirmed, could alter perceptions about how best to exploit the compound in crop protection programmes. The aims of the project described in this report were, therefore, to confirm and extend these initial results and to explore the potential for azoxystrobin to contribute to the practical management of take-all in wheat. It had the following specific objectives:

- (a) To confirm evidence that azoxystrobin applied as a foliar spray can decrease take-all in wheat.
- (b) To seek evidence to help explain the mechanism by which azoxystrobin decreases take-all in wheat.

The results that are presented are mainly from field experiments but include some from a series of pot experiments.

Field Experiments

Data were obtained from a total of six field experiments on winter wheat in harvest years 2000 and 2001. Four of the experiments in 2000 had already been planned by Zeneca (now part of Syngenta) to study effects of strobilurin fungicides on stem base diseases but the company had no plans to measure take-all on the roots. The fungicide treatments that were sampled in the four experiments, which were chosen from among a much larger number of treatments being tested, were selected to study the effects on take-all of azoxystrobin and the newer strobilurin fungicide picoxystrobin. In two of the four experiments (in Suffolk and Lincolnshire), four treatments were selected that compared azoxystrobin (at a single rate) and picoxystrobin (at two rates), applied at the beginning of stem extension, with a treatment that received neither. In the other two experiments (in Wiltshire and Warwickshire), five treatments were selected for sampling that tested combinations of sprays applied early (early stem extension) or late (early booting). The

early sprays were of azoxystrobin (at a single rate) or picoxystrobin (at two rates), and the late sprays were of azoxystrobin (at two rates). In each experiment, there were six replicates of each treatment arranged in fully randomised blocks. Plant samples to measure take-all on the roots were taken in July. The results showed that azoxystrobin applied at the beginning of stem extension significantly decreased the severity of take-all (% plants with moderate or severe symptoms) in three of the four experiments. In contrast, picoxystrobin had no effect.

The fifth experiment, also in 2000, was on a heavy clay soil on the Woburn Experimental Farm of IACR-Rothamsted. It compared azoxystrobin with five related fungicides (kresoxim-methyl, picoxystrobin, pyraclostrobin, trifloxystrobin and an experimental compound that was designated HGCA Z) all applied at 250 g ai/ha. Each was applied twice to the same plots, at the beginning of stem extension and at flag leaf emergence. Plant samples to determine amounts of take-all on the roots were taken from each plot in late June. Severity of take-all on the roots (% plants with moderate or severe disease) was more than halved by azoxystrobin and by HGCA Z, which had very similar (and significant) effects. Plots treated with these two fungicides gave more grain than the untreated plots (+ 15%) and those treated with other fungicides (+ 8%) but the effects on yield were not quite significant.

The final field experiment, in 2001, was intended to determine whether the activity of azoxystrobin against take-all is a reflection of the amount that is deposited on the soil. Plots were sown on a clay loam soil on the Rothamsted Experimental Farm at a single seed rate but with either 12.5 or 25 cm between the rows. Sprays of azoxystrobin (at 250 g ai/ha) were applied at the beginning of stem extension, at flag leaf emergence or at both growth stages to the foliage in plots sown at each of the two row spacings or to the soil in plots sown at the wide row spacing only. There were two replicates of each of the nine fungicide treatments plus one unsprayed plot at each row spacing in each of two fully randomised blocks. Plant samples to determine amounts of take-all on the roots were taken from each plot in early July. Effects of azoxystrobin on take-all in this experiment were, however, very small whether it was applied to the foliage or to the soil. The explanation for the very small effect on take-all in this experiment is uncertain.

Key results

- Azoxystrobin significantly decreased the severity of take-all in four of the six field experiments in which it was tested.
- In the one experiment in which the experimental compound designated HGCA Z was tested, it and azoxystrobin gave very similar decreases in take-all (significant) and increases in yield (not significant).
- None of the other related fungicides that were tested had any significant effects on take-all.

Pot Experiments

Three pot experiments were done to explore in more detail the effects of azoxystrobin, and related compounds, on take-all in wheat. Field soil, naturally infested with take-all, was used in all three.

In the first experiment, azoxystrobin, kresoxim-methyl, picoxystrobin, pyraclostrobin, trifloxystrobin and the experimental compound designated HGCA Z were tested on wheat seedlings grown in a controlled environment. The fungicides were applied to the soil below the seed so that their effects on take-all were not wholly determined by possible differences in their mobility through soil as a consequence of differences in their physico-chemical properties. They were tested at two rates that corresponded on an area basis to 250 or 500 g ai/ha, in six fully randomised blocks. Water was applied to the soil surface. Roots were examined for symptoms of take-all five weeks after sowing. Averaged over the two rates of application, there were similar (and significant) decreases in the severity of take-all (% roots with take-all) following the application of azoxystrobin or HGCA Z. The interaction between fungicides and rates of application was not significant but there tended to be a bigger effect of increasing the rate of application of HGCA Z than of azoxystrobin. There was also evidence of a small decrease in take-all where pyraclostrobin was applied at the larger of the two rates but not at the smaller.

The second of the pot experiments was also done in a controlled environment, and tested the effects on take-all of azoxystrobin and HGCA Z applied to the soil below the seed at rates corresponding on an area basis to 250 or 500 g ai/ha. However, water was applied either to the soil surface or to the plastic saucers in which the pots were stood. The treatments were tested in six randomised blocks, three of which were examined for take-all four weeks after sowing and the remainder eight weeks after sowing. Percentages of plants with take-all after four weeks, and percentages of roots with take-all after four and eight weeks were decreased by azoxystrobin but not by HGCA Z. Azoxystrobin was more effective at the small than at the large rate after both four and eight weeks. HGCA Z tended to increase take-all at the small rate but at the large rate it had no effect (after 4 weeks) or tended to decrease it (after 8 weeks). Effects of top vs bottom watering were inconsistent.

The final pot experiment tested the effects on take-all of azoxystrobin and HGCA Z applied either to the soil surface or to the foliage of winter wheat grown in a glasshouse. A paint brush was used to apply the fungicides to the leaves. They were applied at one of two growth stages (beginning of stem extension or flag leaf emergence) or both, and, after the fungicides had been applied, water was applied either to the surface of the soil or to the plastic saucers in which the pots were stood. The treatments were tested in four randomised blocks and take-all on the roots was measured at anthesis. Azoxystrobin decreased the severity of take-all when applied to the soil but not when applied to the foliage. In contrast, HGCA Z apparently decreased take-all only when applied to the foliage. Such an effect is difficult to explain and should be

viewed with some caution until it has been confirmed. There were no significant effects of altering the timing of fungicide applications (or of applying them twice) or of top vs bottom watering.

Key results

- Take-all was consistently decreased by azoxystrobin applied either to the soil below the seed or to the soil surface.
- Azoxystrobin had no effect on take-all when applied to the foliage in circumstances where contamination of the soil was avoided.
- Effects of the experimental strobilurin HGCA Z were less consistent than the effects of azoxystrobin, and so further testing is needed.

Conclusions and Implications

Data from the field experiments described in this report, together with the limited results previously published, provide convincing evidence that azoxystrobin applied as a foliar spray to winter wheat can give useful decreases in the severity of take-all on the roots. However, the magnitude (and significance) of the effects differed between experiments. In the previously-reported work, the effects of azoxystrobin on take-all differed between cultivars but not consistently. The value of azoxystrobin as a tool in the practical management of take-all may, therefore, be limited unless and until the reasons for this variability are understood. In the meantime, effects on take-all may best be seen as a potential bonus in situations where applications of azoxystrobin, at the rates and times at which it has so far mostly been tested and been shown to be effective against take-all (i.e. 250 g ai/ha at the beginning of stem extension), can be justified by its predicted effects on other diseases. There was no evidence of effects against take-all of the related compounds that were tested and that are commercially available, some of which may currently be applied more commonly than azoxystrobin at the beginning of stem extension. Sprays of an experimental compound had similar effects on take-all to azoxystrobin in the one field experiment in which it was tested but results from pot experiments were equivocal and so further tests are needed.

TECHNICAL DETAIL

GENERAL INTRODUCTION

The wheat take-all fungus (*Gaeumannomyces graminis* var. *tritici*) is, like all soil-borne, root-infecting fungi, a difficult target for fungicidal control. Although a number of well-established commercial fungicides are active against the pathogen and have been shown to decrease the disease in field experiments at Rothamsted (Bateman, 1989), the amounts and methods of application (e.g. high volume soil drenches) that are needed, make their use uneconomic. Management of take-all has, therefore, relied largely on appropriate husbandry (Hornby *et al.*, 1998).

Recently, however, two fungicides, fluquinconazole (Wenz *et al.*, 1998; Löchel *et al.*, 1998) and silthiofam (Beale *et al.*, 1998; Spink *et al.*, 1998), which have useful activity against take-all when applied to the seed, have become available. Both provide only partial control of the disease but, when used as part of an integrated disease management package, offer farmers the prospect of better disease control and greater flexibility in cropping.

Applying crop protection chemicals to the seed has a number of advantages. In particular, it allows their use in small amounts, and often helps to minimise the risk to non-target organisms but they offer little flexibility in the amounts that can be applied and no flexibility in the timing of applications, in contrast to foliar sprays. However, the discovery of agrochemicals with the appropriate properties to move systemically from the leaves to the roots has proved very difficult. Amounts of chemical moving to the soil externally, as a result of run-off, can usually be expected to be relatively small because formulations are designed to adhere to the leaves and to be rain-fast. Consequently, a cautious view was taken of evidence from an experiment at IACR-Rothamsted in 1998 that suggested significant decreases in take-all where azoxystrobin had been applied as a foliar spray. Occasional effects on take-all of fungicide sprays (or programmes of sprays) have been seen previously (e.g. Prew *et al.*, 1995) but have, typically, proved impossible to repeat. However, further experiments in 1999, including one at Harper Adams University College, showed similar effects of azoxystrobin on take-all (Jenkyn *et al.*, 2000) suggesting that it was a real effect although the magnitude of the effect differed between experiments. There were also interactions with cultivars that were significant but inconsistent.

Although azoxystrobin is known to be active against *Gaeumannomyces* spp. (e.g. Soika & Sanders, 1996), its effects on take-all in the experiments reported by Jenkyn *et al.* (2000) are difficult to explain from its predicted behaviour in plants and soil. Such effects may, nevertheless, contribute to yield responses in crops

at risk from the disease and, if confirmed, could alter perceptions about how best to exploit the compound in crop protection programmes.

Whether azoxystrobin has the potential to contribute more specifically to the practical management of take-all, perhaps in combination with the new seed treatment fungicides, is uncertain. The inconsistent effects on different cultivars would, for example, make it difficult to exploit the fungicide efficiently unless they could also be predicted. Progress toward this is unlikely without a better understanding of the mechanism by which azoxystrobin decreases take-all.

The objects of the project described in this report were, therefore, to confirm and extend the initial findings reported by Jenkyn *et al.* (2000), and to explore the potential for azoxystrobin to contribute to the practical management of take-all in wheat. It had the following specific objectives:

- (a) To confirm evidence that azoxystrobin applied as a foliar spray can decrease take-all in wheat.
- (b) To seek evidence to help explain the mechanism by which azoxystrobin decreases take-all in wheat.

EXPERIMENTAL

PART 1: FIELD EXPERIMENTS

Introduction

The results presented in this part of the report are from a total of six field experiments in harvest years 2000 and 2001. Four of the experiments (Experiments 1.1 - 1.4, in 2000) had already been planned by Zeneca (now part of Syngenta) to study effects of strobilurin fungicides on stem base diseases of wheat but the company had no plans to measure take-all on the roots. The other two experiments were specifically designed for the purposes of this project and were sited on the IACR experimental farms at Woburn in 2000 and Rothamsted in 2001 (Experiments 1.5 and 1.6, respectively).

For Experiments 1.1 - 1.4 the only information made available to the authors of this report, apart from details of the treatments tested, concern cultivars and sowing dates, and the fungicides that were applied to all plots to minimise differences in diseases apart from those that the experiments were designed to study. Similar information is provided for the experiments on the Woburn and Rothamsted Experimental Farms. Other details concerning the management of the latter experiments, such as the cultivations used to prepare the seedbeds, and applications of herbicides, molluscicides and insecticides that were made to all plots, are omitted. However, this information, should it be required, is readily available from the records kept at IACR-Rothamsted.

Materials and Methods

Experiments 1.1 - 1.4

The four Zeneca experiments that were sampled were in Suffolk, Lincolnshire, Wiltshire and Warwickshire (Experiments 1.1 - 1.4, respectively). The cultivars (and sowing dates) on the four sites were, respectively, Claire (14 - 17 October 1999; exact sowing date for the area occupied by the experiment not known), Soissons (15 October 1999), Riband (12 October 1999) and Charger (26 October 1999). The site used for Experiment 1.3 had been in continuous wheat for at least 12 years but the other three sites were growing second (Experiment 1.4) or third (Experiments 1.1 and 1.2) wheats after a break.

The fungicide treatments that were sampled in the four experiments, which were chosen from among a much larger number of treatments being tested, were selected to study the effects on take-all of azoxystrobin and the newer strobilurin fungicide picoxystrobin. In Experiments 1.1 and 1.2, which were designed to test the effects of fungicides on sharp eyespot (*Rhizoctonia cerealis*), four treatments were selected that compared azoxystrobin and picoxystrobin, applied at the beginning of stem extension, with a treatment that received

neither (Table 1). Dates (and growth stages; Zadoks, Chang & Konzak, 1974) when these treatments were applied in Experiments 1.1 and 1.2 were 5 May (GS 30-31) and 16 April (GS 31), respectively. All of the sampled plots in these two experiments were subsequently sprayed with a formulated mixture of the fungicides epoxiconazole and fenpropimorph.

Table 1. Details of the strobilurin fungicides tested and their rates of application (g ai/ha) in Experiments 1.1 - 1.4¹.

Treatment No.	Expts. 1.1 and 1.2	Experiments 1.3 and 1.4	
	Early spray	Early spray	Late spray ²
1	None	None	None
2	Azoxystrobin (250)	None	Azoxystrobin (200) + E
3	Picoxystrobin (250)	Azoxystrobin (250)	Azoxystrobin (250)
4	Picoxystrobin (500)	Picoxystrobin (250)	Azoxystrobin (200) + E
5	-	Picoxystrobin (500)	Azoxystrobin (200) + E

¹ Early sprays were applied at the beginning of stem extension and late sprays at early booting; see text for further details.

² E = epoxiconazole at 62.5 g ai/ha.

In Experiments 1.3 and 1.4, which were designed to test the effects of fungicides on eyespot (*Tapesia* spp.), the five treatments that were selected for sampling tested combinations of sprays applied early (early stem extension) and late (early booting). The early sprays were of azoxystrobin or picoxystrobin, and the late sprays were of azoxystrobin alone or in combination with epoxiconazole. Details of the treatments are provided in Table 1 but because epoxiconazole was not expected to affect take-all it has, for simplicity, been omitted from the tables of results. The early and late sprays in Experiment 1.3 were applied on 14 April and 30 May (GS 30 and GS 43, respectively) and those in Experiment 1.4 on 10 April and 29 May (details of actual growth stages when sprayed, not supplied). All of the sampled plots in Experiments 1.3 and 1.4 were sprayed with the fungicide pencycuron in March, before the treatment sprays were applied.

Plots were 3m wide and at least 17m long but sampling to measure take-all was confined to an area 3m wide x 5m long at one end of each plot. There were six replicates of each treatment arranged in fully randomised blocks. Small samples of plants were collected from each of the four sites in late April or early May, and sent to Rothamsted to confirm the presence of take-all on the roots before deciding to proceed with more

detailed sampling in July. Five 20 cm lengths of row were sampled from each plot on 14, 11, 17 and 13 July, respectively, in Experiments 1.1 - 1.4 (GS 77 except GS 83 in Experiment 1.3).

Experiment 1.5

This experiment compared azoxystrobin with five related fungicides including an experimental compound that was designated HGCA Z. It was on a heavy clay soil on the Woburn Experimental Farm and followed winter wheat in 1998 and 1999. Plots (each 3 m wide x 10 m long) were sown with winter wheat (cv. Consort, treated with bitertanol + fuberidazole) at 300 seeds/m² on 16 September 1999. The fungicides (azoxystrobin, kresoxim-methyl, picoxystrobin, pyraclostrobin, trifloxystrobin and HGCA Z) were all applied at 250 g ai in 220 l water/ha. Each was applied twice to the same plots, on 27 April (c. GS 31-32) and 20 May (c. GS 39). There were four replicate plots of each of the six fungicide treatments, arranged in four fully randomised blocks, and two untreated plots per block. Fungicide sprays to minimise differences in diseases apart from take-all were applied to all plots on 30 April (epoxiconazole) and 20 May (epoxiconazole + chlorothalonil). Other applications (which included nitrogen at 150 kg/ha on 2 May) conformed to standard practice on the Woburn Experimental Farm.

Plant samples to determine amounts of take-all on the roots were taken from each plot on 22 June (GS 69; 10 x 20 cm lengths of row/plot). Grain yields were measured after combine harvesting the plots on 18 August.

After harvesting the experiment, the site was ploughed (on 21 September 2000) and the plots re-sown with winter wheat (cv. Claire, treated with bitertanol + fuberidazole) at 375 seeds/m² on 5 October, to determine whether there were any residual effects of the treatments on take-all in 2001. Management of the crop conformed to standard practice on the Woburn Experimental Farm. Samples of plants were taken from the plots on 3 April (GS 22 - 23; 5 x 15 cm lengths of row/plot) and 20 June (GS 69; 5 x 20 cm lengths of row/plot).

Experiment 1.6

This experiment was designed to study the mechanism by which azoxystrobin applied to the leaves affects take-all on the roots, and, in particular, whether the activity of the fungicide against take-all is a reflection of the amount that is deposited on the soil.

The experiment was on a flinty clay loam soil on the Rothamsted Experimental Farm, and followed winter wheat in 1999 and 2000. Plots of winter wheat (cv. Hereward, treated with bitertanol + fuberidazole) were sown at 350 seeds/m² on 20 October 2000 with either 12.5 or 25 cm between the rows. Sprays of azoxystrobin (at 250 g ai/ha) were applied on 11 May (GS 32; spring), 31 May (GS 39; summer) or on both

dates to the foliage in plots sown at each of the two row spacings or to the soil in plots sown at the wide row spacing only. Sprays to the foliage were applied in 220 l water/ha using a small-plot hydraulic sprayer that consisted of a hand-held boom (fitted with flat fan nozzles, 02-110-20), connected to a back-pack that propelled the solution using compressed air. Similar equipment, fitted with drop legs, was used to apply sprays to the soil but different (02-Pruejet) nozzles were used that generated larger droplets, so that deposition on the soil was maximised and drift on to the adjacent foliage was minimised. Twice as many nozzles were fitted to the equipment used to apply sprays to the soil than to that used to apply sprays to the foliage because there was one drop leg (and one nozzle) between each pair of rows. Sprays to the soil were, therefore, applied in twice the volume (440 l water/ha) but at half the concentration of those applied to the foliage.

There were two replicates of each of the nine fungicide treatments plus one unsprayed plot at each row spacing in each of two fully randomised blocks. To minimise differences in diseases apart from take-all, all plots were sprayed on 15 May with epoxiconazole plus cyprodinil and on 6 June with epoxiconazole. Other applications to the experiment, including nitrogen at 60 kg/ha on 27 March and 150 kg/ha on 4 May, conformed to standard practice on the Rothamsted Experimental Farm.

Plant samples to determine amounts of take-all on the roots were taken from each plot on 11 July (GS 73; 10 x 20 cm lengths of row/plot). Grain yields were measured after combine harvesting the plots on 1 August.

Disease assessments

Plants for assessing take-all on the roots were dug from the plots using a fork. The roots were washed free of soil and either assessed immediately (samples taken in April or May) or air dried and then stored in an unheated glasshouse for assessment at a later date (samples taken in June or July). Roots on the early samples were examined under water in a white dish, and the numbers of plants and roots with take-all recorded. After re-wetting, root systems on the later samples were examined in a similar way and take-all assessed as slight1 (<10% roots infected), slight 2 (11 - 25%), moderate 1 (26 - 50%), moderate 2 (51 - 75%) or severe (> 75%). The data were used to calculate a take-all index for each plot [(% plants with infection in the slight 1 category + 2 x % slight 2 + 3 x % moderate 1 + 4 x % moderate 2 + 5 x % severe) ÷ 5; maximum = 100]. To help interpret yields, plants sampled in June or July were also examined to determine amounts of the stem base diseases eyespot (*Tapesia* spp.), sharp eyespot (*Rhizoctonia cerealis*) and brown foot rot (*Fusarium* spp.) but these data from Experiments 1.1 - 1.4 are omitted because yield data were not made available. A logit transformation was used for the analysis of data expressed as percentages. The tables show the mean logit values for individual treatments and the appropriate standard errors of differences between them. Percentages obtained by back transformation are shown in parentheses.

Results

Experiment 1.1

In plots sprayed with azoxystrobin, there were fewer plants with take-all, and especially with symptoms that were moderate or severe, than in plots sprayed with picoxystrobin or neither (Table 2). Overall, differences between the four treatments were not significant. However, the contrast between azoxystrobin and the mean of the other three treatments (with 1 DF) was significant for percentage plants with moderate or severe symptoms (P (logit transforms) = 0.03) and for take-all index (P = 0.03).

Table 2. Effects of azoxystrobin and picoxystrobin on the incidence and severity of take-all in Experiment 1.1¹

Fungicide treatment² (g ai/ha)	Take-all		
	Total (% plants)	Moderate + severe (% plants)	Index
None	+0.70 (80.3)	-0.41 (30.7)	34.0
Azoxystrobin (250)	+0.36 (67.0)	-0.89 (14.4)	23.6
Picoxystrobin (250)	+0.64 (78.3)	-0.57 (24.4)	32.4
Picoxystrobin (500)	+0.63 (78.0)	-0.57 (24.3)	31.6
SED (15 DF)	0.182	0.187	4.72
Probability	0.26	0.11	0.17

¹Data (except take-all indices) are logit transform values with, in parentheses, the corresponding percentage values obtained by back transformation.

²Fungicide treatments were applied at the beginning of stem extension; see text for further details.

Experiment 1.2

Take-all was common on the roots of plants sampled from this experiment but most of the symptoms were slight. Incidence of the disease (% plants) was significantly decreased by azoxystrobin (judged by the contrast between this treatment and the mean of the other three; P (logit transforms) = 0.02) but effects on severity (% plants with moderate or severe symptoms) and, as a consequence of this, on take-all index were, relatively, much larger and more significant (Table 3).

Table 3. Effects of azoxystrobin and picoxystrobin on the incidence and severity of take-all in Experiment 1.2.¹

Fungicide treatment ² (g ai/ha)	Take-all		
	Total (% plants)	Moderate + severe (% plants)	Index
None	+0.83 (84.1)	-1.12 (9.7)	23.8
Azoxystrobin (250)	+0.43 (70.4)	-2.13 (1.4)	14.6
Picoxystrobin (250)	+0.85 (84.5)	-0.69 (20.2)	29.5
Picoxystrobin (500)	+1.16 (91.0)	-1.00 (11.9)	27.7
SED (15 DF)	0.244	0.169	2.67
Probability	0.07	<0.001	<0.001

¹Data (except take-all indices) are logit transform values with, in parentheses, the corresponding percentage values obtained by back transformation.

²Fungicide treatments were applied at the beginning of stem extension; see text for further details.

Experiment 1.3

Differences between treatments in the incidence of take-all were significant, and indicated that the disease was least common where two sprays of azoxystrobin had been applied or, less explicably, where azoxystrobin followed picoxystrobin at the larger of the two rates tested (Table 4). Differences in severity (% plants with moderate or severe symptoms) were more straightforward and showed a modest (and not significant) decrease where a single spray of azoxystrobin (at 200 g ai/ha) was applied at early booting but a much larger (and significant) effect where a spray of azoxystrobin at this growth stage (at 250 g ai/ha) followed an application of the same fungicide at the beginning of stem extension (probabilities based on the contrast between none *vs* one *vs* two sprays of azoxystrobin were 0.03 and 0.02 for logits of percentage plants with moderate or severe symptoms and for take-all indices, respectively).

Table 4. Effects of azoxystrobin and picoxystrobin on the incidence and severity of take-all in Experiment 1.3¹

Fungicide treatment ² (g ai/ha)		Take-all		
		Total (% plants)	Moderate + severe (% plants)	Index
Early	Late			
None	None	+1.43 (94.6)	-0.30 (35.7)	42.0
None	Azoxy (200)	+1.43 (94.5)	-0.50 (26.9)	37.5
Azoxy (250)	Azoxy (250)	+0.92 (86.4)	-0.93 (13.5)	26.9
Picoxy (250)	Azoxy (200)	+1.13 (90.6)	-0.47 (28.0)	34.7
Picoxy (500)	Azoxy (200)	+0.87 (85.2)	-0.59 (23.5)	34.5
SED (20 DF)		0.195	0.225	4.76
Probability		0.02	0.11	0.06

¹Data (except take-all indices) are logit transform values with, in parentheses, the corresponding percentage values obtained by back transformation.

²Early sprays were applied at the beginning of stem extension and late sprays at early booting; see text for further details. Azoxy = azoxystrobin, Picoxy = picoxystrobin.

Experiment 1.4

On average, there were fewer plants infected with take-all where picoxystrobin had been applied at the beginning of stem extension than where azoxystrobin or nothing had been applied at the same growth stage (Table 5) but plants with moderate or severe symptoms were least common where azoxystrobin was applied (and was followed by a second spray of the same fungicide at early booting). However, the differences were small and none of them was significant.

Table 5. Effects of azoxystrobin and picoxystrobin on the incidence and severity of take-all in Experiment 1.4¹

Fungicide treatment ² (g ai/ha)		Take-all		
		Total (% plants)	Moderate + severe (% plants)	Index
Early	Late			
None	None	+0.56 (75.2)	-0.75 (18.3)	31.0
None	Azoxy (200)	+1.08 (89.6)	-0.80 (16.8)	31.9
Azoxy (250)	Azoxy (250)	+0.89 (85.6)	-0.89 (14.4)	30.1
Picoxy (250)	Azoxy (200)	+0.64 (78.2)	-0.69 (20.1)	29.2
Picoxy (500)	Azoxy (200)	+0.56 (75.5)	-0.65 (21.6)	29.3
SED (20 DF)		0.26	0.337	5.96
Probability		0.21	0.96	0.99

¹Data (except take-all indices) are logit transform values with, in parentheses, the corresponding percentage values obtained by back transformation.

²Early sprays were applied at the beginning of stem extension and late sprays at early booting; see text for further details. Azoxy = azoxystrobin, Picoxy = picoxystrobin.

Experiment 1.5

Most of the plants sampled from this experiment (in June) were affected by take-all and differences between the treatments in incidence of the disease were relatively small and not significant (Table 6). There were, however, significantly fewer plants with moderate or severe disease in plots treated with azoxystrobin or HGCA Z than in those treated with the other fungicides or none. The mean for these two treatments (24.6%) was less than half that for all other plots (57.5 %) and there were corresponding, and significant, effects on take-all index.

Eyespot was the commonest of the stem base diseases, affecting, on average, 38.7% of straws. However, symptoms were mostly slight, and differences between the treatments were small and not significant (Table 6). On average, sharp eyespot affected only 3.2% of straws, and brown foot rot only 6.6%.

Overall, the differences between treatments in grain yield were not quite significant (Table 6). Plots treated with azoxystrobin or HGCA Z (which had least take-all) did, however, give more grain than the untreated plots (+ 1.13 t/ha; + 15.4%) and those treated with the other fungicides (+0.64 t/ha; + 8.2%).

Measurements of take-all on the roots of the wheat grown in the plots in 2001 provided no evidence to indicate residual effects of the treatments applied to the previous crop, in 2000.

Table 6. Effects of strobilurin fungicides on take-all, eyespot and grain yield in Experiment 1.5¹.

Fungicide treatment ²	Take-all			Eyespot	Grain yield (t/ha)	
	Total (% plants)	Moderate+ severe (% plants)	Index	Total (% straws)		
None	+1.80 (97.3)	+0.21 (60.4)	53.7	-0.17 (41.7)	7.31	
Azoxystrobin	+1.64 (96.4)	-0.52 (26.2)	35.7	-0.08 (45.9)	8.42	
Kresoxim-methyl	+1.84 (97.6)	+0.05 (52.6)	50.3	-0.13 (43.3)	7.69	
Picoxystrobin	+2.04 (98.3)	+0.40 (68.8)	56.5	-0.25 (37.6)	7.51	
Pyraclostrobin	+1.34 (93.5)	-0.03 (48.5)	46.2	-0.30 (35.6)	7.94	
Trifloxystrobin	+1.53 (95.5)	+0.07 (53.6)	50.5	-0.29 (36.0)	8.03	
HGCA Z	+1.41 (94.4)	-0.60 (23.1)	35.1	-0.46 (28.4)	8.45	
SED ³ (22 DF)	1	0.244	0.294	6.76	0.163	0.451
	2	0.211	0.254	5.86	0.141	0.391
Probability	0.07	0.02	0.02	0.31	0.06	

¹Data (except take-all indices and grain yields) are logit transform values with, in parentheses, the corresponding percentage values obtained by back transformation.

²Each of the fungicides was applied twice, at the beginning of stem extension and at flag leaf emergence; see text for further details.

³SED 1 is for comparisons between the fungicides, and SED 2 for comparing any of the fungicides with none.

Experiment 1.6

Take-all, measured in July, was less severe than expected in a third consecutive wheat crop probably as a result of relatively late sowing (20 October) which was a consequence of the wet and very difficult conditions in autumn 2000. On average, 72.1% of plants were affected by the disease and 15.7% had symptoms that were moderate or severe.

There was a significant effect of spray timing on the incidence of take-all (% plants), with less disease following the spring (or spring + summer) application of azoxystrobin than after the summer application alone. Applying the fungicide to the soil or to the foliage in plots sown at different row spacings had no

significant effect. Results from the extra plots, which were not sprayed with azoxystrobin, suggested, however, that the incidence of take-all was greater, albeit not significantly, in plots sown at the wide than at the narrow row spacing. Accordingly, the mean values for unsprayed plots in Table 7 are weighted to recognise the different proportions of sprayed plots in the factorial part of the experiment that were sown at the narrow and wide row spacings (i.e. 1 : 2). These show that the average decreases in take-all achieved in this experiment were, despite the significant effects of spray timing, modest compared to those seen in some earlier experiments.

Numbers of straws affected by eyespot, brown foot rot and sharp eyespot in the plots sprayed with azoxystrobin averaged 18.4, 13.1 and 1.6%, respectively, but symptoms were mostly slight and unaffected by the timing of sprays or by row-spacing and methods of application. There were, similarly, no significant effects on grain yield (Table 7).

Table 7. Effects of azoxystrobin sprays applied at different times on the incidence and severity of take-all and on grain yield in Experiment 1.6¹.

Azoxystrobin sprays ²	Take-all			Grain yield (t/ha)
	Total (% plants)	Moderate + severe (% plants)	Index	
None	+0.52 (73.8)	-0.70 (19.8)	27.2	6.57
Spring	+0.44 (70.6)	-0.88 (14.7)	23.4	6.46
Summer	+0.71 (80.4)	-0.62 (22.3)	31.0	6.51
Spring + summer	+0.28 (63.8)	-0.94 (13.2)	22.2	6.53
SED ³ (33 DF)	1	0.136	0.164	0.323
	2	0.192	0.232	0.456
Probability ⁴	0.01	0.14	0.02	0.97

¹Data (except take-all indices and grain yields) are logit transform values with, in parentheses, the corresponding percentage values obtained by back transformation.

²Spring sprays were applied at GS 32, and summer sprays at GS 39; see text for further details.

³SED 1 is for comparisons between the treatments testing spray times, and SED 2 for comparing any of these with none.

⁴The probabilities that the differences between spray times, excluding none, occurred by chance.

EXPERIMENTAL

PART 2: POT EXPERIMENTS

Introduction

In this part of the report, three pot experiments are described that sought to explore in more detail the effects of azoxystrobin, and related compounds, on take-all in wheat. The first of these (Experiment 2.1) tested the same fungicides that were tested in the field in Experiment 1.5. The other two pot experiments tested the two fungicides for which there was most evidence of significant activity (azoxystrobin and HGCA Z). In Experiments 2.1 and 2.2, the fungicides were applied to the soil but placed below the seed so that their effects on take-all were not wholly determined by possible differences in their mobility through soil as a consequence of their different physico-chemical properties. In Experiment 2.3, the fungicides were applied to the surface of the soil or to the foliage.

Materials and Methods

Experiment 2.1

In this experiment, the effects on take-all of azoxystrobin, kresoxim-methyl, picoxystrobin, pyraclostrobin, trifloxystrobin and the experimental compound designated HGCA Z were tested on wheat seedlings grown in a controlled environment. Each fungicide was tested at two rates that corresponded on an area basis to 250 and 500 g ai/ha except that, due to an error, kresoxim-methyl was only tested at 250 g ai/ha. These treatments were compared with another in which no fungicide was applied.

Clay loam soil, naturally infested with take-all, was collected from a field on the Rothamsted Experimental Farm, crumbled to an even texture, and large stones removed. The soil (200 cm³/pot) was then placed in 11 cm tall white plastic pots (drinking cups; obtained from Mono Containers Ltd., ref. PV11) previously drilled with four 3 mm diameter drainage holes and each containing 50 cm³ of course sand. Appropriate stock solutions of each of the fungicides were prepared, using the formulated materials, and applied to the surface of the soil at 5 or 10ml/pot, to achieve the two rates of application of active ingredient. Another 50 cm³ of soil was then added to each pot before sowing germinated seeds of wheat (cv. Hereward) at 8 per pot. The seeds were covered with a layer of Lee Moor elutriated sand (Cornish grit). There were six replicate pots of each of the fungicide treatments, arranged in six fully randomised blocks, and two untreated pots/block. After sowing, the pots were transferred to a controlled environment room set to run with a 16 h day, and 15/10°C day/night temperatures. Pots were stood in plastic saucers, and the soil watered from above. Five weeks after sowing, the root ball was knocked out of the pots, and the roots trimmed at the lower soil/sand interface. Roots were washed free of soil and examined under water in a white dish for take-all lesions.

Total numbers of plants and roots (main axes), and the numbers affected by take-all were recorded, and used to calculate percentages of plants and roots with symptoms of the disease.

Experiment 2.2

In this experiment, the effects on take-all of azoxystrobin and HGCA Z were tested using similar methods to those described for Experiment 2.1. However, the experiment also compared a clay loam soil collected from a field on the Rothamsted Experimental Farm and a sandy loam soil collected from a field on the Woburn Experimental Farm. The fungicides were applied to the soil below the seed at rates corresponding on an area basis to 250 or 500 g ai/ha. When watering, measured amounts were applied either to the soil surface or to the plastic saucers in which the pots were stood. There were six replicates of each of the 16 (2^4) treatment combinations testing the fungicides, arranged in six randomised blocks, plus two pots per block that were given no fungicide and that tested the effects of each of the two soils and top vs bottom watering. Roots on seedlings growing in pots from three of the blocks were assessed for take-all four weeks after sowing and those in the remaining blocks, eight weeks after sowing.

Experiment 2.3

This experiment tested the effects on take-all of azoxystrobin and HGCA Z applied to the soil or to the foliage of winter wheat grown in a glasshouse. The fungicides were applied at one of two growth stages or at both, and the soil was either top- or bottom- watered.

Clay loam soil, naturally infested with take-all, was collected from a field on the Rothamsted Experimental Farm, crumbled to an even texture, and large stones removed. The prepared soil was placed in 13 cm pots (c. 1100 g of soil/pot), before placing 5 germinated seeds of winter wheat (cv. Hereward) on the surface of the soil in each pot. The seeds, which were sown on 26-27 October, were then covered with a further 70 g of the prepared soil. Pots were watered after sowing and, thereafter, measured volumes of water were applied as necessary to restore them to a standard weight (c. 1300 g/pot initially but 1350 g/pot after the first of the fungicide treatments had been applied). Prior to the application of the fungicide treatments, water was applied to the surface of the soil.

The fungicides were applied at a rate that corresponded, on an area basis, to 250 g ai/ha. Solutions were prepared at concentrations that delivered the required amounts of active ingredient in 5 ml of water, for application to the soil surface, or 250 μ l of water, for application to the leaves. The required volumes were pipetted directly onto the soil surface or into watch glasses from which they were transferred to the undersides of the top two fully-expanded leaves on each shoot using a fine paint brush. A different brush was used for each fungicide, and they were wetted (using the same stock solutions) before being used to

apply the treatments. Fungicides were applied on 4-5 April (GS 31), 2-3 May (GS 39) or on both dates. After applying the first of the fungicide treatments, water was applied either to the soil surface or into the plastic saucers in which each of the pots was placed. There were four replicates of each of the 24 fungicide treatments, arranged in four randomised blocks, and two pots/block given no fungicides that tested the effects of each of the two watering regimes.

At anthesis (1 June; GS 69), the roots were washed to remove the soil, and examined under water in a white dish. Take-all symptoms were assessed by counting the roots and those affected by the disease, as described for the other pot experiments (Experiments 2.1 and 2.2). The overall severity of the symptoms on each root system was also graded, as described for the field experiments, and, from these, take-all indices were calculated.

Data analysis

As for the field experiments, a logit transformation was used for the analysis of data expressed as percentages. The tables show the mean logit values for individual treatments and the appropriate standard errors of differences between them. Percentages obtained by back transformation are shown in parentheses.

Results

Experiment 2.1

Although most of the seedlings in this experiment were affected by take-all, there were, on average, small and significant decreases associated with the application of azoxystrobin or HGCA Z (Table 8). There were similar, but much larger and more significant, effects of these two fungicides on numbers of infected roots/plant (a measure of severity). However, all fungicides except trifloxystrobin significantly decreased the total number of roots/plant (main axes of seminal plus crown roots), and azoxystrobin and HGCA Z were the two that had the largest effects (a mean of 5.4 roots/plant compared with 6.7 roots/plant in the untreated pots). As this could have distorted the results, Table 8 shows percentages of roots with take-all instead of numbers.

Azoxystrobin and HGCA Z both significantly decreased severity of take-all (i.e. % roots infected) at the smaller of the two rates. The interaction between fungicides and rates of application was not significant but there were indications that there were much bigger effects of increasing the rate of application of HGCA Z than of azoxystrobin. There was a small decrease in severity of take-all where pyraclostrobin was applied at the larger of the two rates tested but this was not significant.

Table 8. Effects of fungicides, applied at rates equivalent to 250 or 500 g ai/ha (on an area basis), on incidence and severity of take-all on seedlings of wheat grown in naturally-infested soil in Experiment 2.1¹

Fungicide	% plants with take-all			% roots with take-all		
	250	500	Mean	250	500	Mean
None	+1.42 (94.4)		+1.42 (94.4)	+0.09 (54.7)		+0.09 (54.7)
Azoxystrobin	+1.10 (90.0)	+1.32 (93.3)	+1.21 (91.8)	-0.28 (36.5)	-0.36 (32.6)	-0.32 (34.5)
Kresoxim-methyl	+1.39 (94.1)	-	-	+0.16 (57.9)	-	-
Picoxystrobin	+1.41 (94.3)	+1.41 (94.3)	+1.41 (94.3)	+0.25 (62.3)	+0.24 (61.7)	+0.25 (62.0)
Pyraclostrobin	+1.41 (94.3)	+1.41 (94.3)	+1.41 (94.3)	+0.21 (60.2)	-0.04 (47.9)	+0.08 (54.0)
Trifloxystrobin	+1.41 (94.3)	+1.32 (93.3)	+1.36 (93.8)	+0.18 (59.0)	+0.14 (56.8)	+0.16 (57.9)
HGCA Z	+1.42 (94.4)	+1.12 (90.3)	+1.27 (92.6)	-0.16 (42.0)	-0.55 (24.9)	-0.36 (32.9)
SED ² (61DF) 1	0.112		0.079	0.119		0.084
2	0.097		0.079	0.103		0.084
Probability ³	0.04		0.05	0.14		<0.001

¹Data are logit transform values with, in parentheses, the corresponding percentage values obtained by back transformation.

²SED 1 is for comparisons between the fungicide treatments, and SED 2 for comparing any of these with none.

³The probabilities that differences between the fungicides (except kresoxim-methyl), excluding none, and their interactions with rates of application, occurred by chance.

Experiment 2.2

Take-all was more severe on the roots of seedlings grown in the Woburn soil than on those grown in the Rothamsted soil (e.g. 17.0 vs 10.1% roots infected after 4 weeks and 50.3 vs 32.8% after 8 weeks). However, the results presented in the tables are averages for the two soils because the effects of the other treatments were mostly similar in each of them.

After four weeks there were significantly fewer roots/plant (main axes of seminal plus crown roots) in the Rothamsted than in the Woburn soil (6.2 and 6.6, respectively), and in pots subjected to the top- instead of the bottom-watering regime (6.1 and 6.7, respectively). There were also significantly fewer roots/plant where HGCA Z had been applied (6.1) than where azoxystrobin or neither had been applied (6.5). Effects after 8 weeks were more complex. Where no fungicides had been applied there were fewer roots/plant in pots subjected to the bottom- instead of the top-watering regime (cf results after 4 weeks). The average

effect of the two fungicides was to decrease significantly the number of roots (7.7 vs 8.0) but they only did so in pots subjected to the top-watering regime (7.5 vs 8.3 roots/plant) and HGCA Z did not do so in the Woburn soil. Because of these differences in total numbers of roots, Table 9 shows percentages of roots with take-all rather than numbers.

Table 9. Effects of azoxystrobin and HGCA Z, applied at rates equivalent to 250 or 500 g ai/ha (on an area basis), on incidence and severity of take-all on seedlings of wheat grown in naturally-infested soil in Experiment 2.2¹.

Fungicide	% plants with take-all			% roots with take-all		
	250	500	Mean	250	500	Mean
After 4 weeks						
None	+0.83(84.0)		+0.83(84.0)	-0.90(14.3)		-0.90(14.3)
Azoxystrobin	+0.41(69.4)	+0.62(77.7)	+0.52(73.8)	-1.34(6.4)	-1.02(11.4)	-1.18(8.6)
HGCA Z	+0.99(87.9)	+0.83(84.1)	+0.91(86.1)	-0.63(22.0)	-0.88(14.7)	-0.76(18.0)
SED ² (50 DF) 1	0.200		0.142	0.128		0.090
2	0.174		0.142	0.110		0.090
Probability ³	0.19		0.008	0.003		<0.001
After 8 weeks						
None	+1.35(93.7)		+1.35(93.7)	-0.08(46.1)		-0.08(46.1)
Azoxystrobin	+1.27(92.6)	+1.21(91.8)	+1.24(92.2)	-0.49(27.4)	-0.29(35.7)	-0.39(31.4)
HGCA Z	+1.38(94.0)	+1.32(93.3)	+1.35(93.6)	+0.14(56.9)	-0.26(37.3)	-0.06(47.0)
SED ² (50 DF) 1	0.113		0.080	0.129		0.091
2	0.098		0.080	0.111		0.091
Probability ³	0.97		0.19	0.002		<0.001

¹Data are logit transform values with, in parentheses, the corresponding percentage values obtained by back transformation.

²SED 1 is for comparisons between the fungicide treatments, and SED 2 for comparing any of these with none.

³The probabilities that differences between the fungicides, excluding none, and their interactions with rates of application, occurred by chance.

None of the treatments had any significant effect on percentages of plants with take-all after 8 weeks but this is probably because, by that time, most of the plants had become infected by the disease (mean = 93.2%). In contrast, percentages of plants with take-all after four weeks, and percentages of roots with take-all after four and eight weeks were, on average, decreased significantly by azoxystrobin but not by HGCA Z (Table 9).

These effects were apparently modified by other treatments. Thus the effect of azoxystrobin on percentages of plants with take-all after four weeks was only apparent with the top-watering regime ($P = 0.04$), and after eight weeks the apparently different effects of the two fungicides on percentages of roots with take-all were more evident in the Woburn than in the Rothamsted soil ($P = 0.02$). However, because the interactions were inconsistent (e.g. between sampling dates) and made no obvious biological sense, they are thought to be spurious.

There were also significant effects of fungicide rates on percentages of roots with take-all but they were different for the two fungicides (Table 9). After four weeks, azoxystrobin was apparently more effective at the small than at the large rate whereas HGCA Z tended to increase take-all at the small but not at the large rate. For both fungicides, these effects of rate were larger with bottom than with top watering ($P = 0.05$). After eight weeks, azoxystrobin was again more effective at the small than at the large rate (especially with bottom watering). In contrast, HGCA Z tended to increase take-all (compared to the untreated) at the small rate but to decrease it at the large rate (especially with top watering); the difference between the two rates was significant.

Experiment 2.3

By the time that the plants in this experiment were examined for take-all, most of them were affected by the disease (Table 10), and there was little evidence of significant differences between the treatments. The mean differences between the fungicides and the untreated in percentages of plants with moderate or severe symptoms (and in take-all index) were not significant (Table 10) but there was strong evidence of a significant interaction between the fungicides and the method (site) of application (i.e. soil vs foliage). This suggested that azoxystrobin decreased the severity of take-all only when applied to the soil whereas HGCA Z decreased take-all only when applied to the foliage. There were no significant effects of altering the timing of fungicide applications (or of applying them twice) or of top vs bottom watering.

Table 10. Effects of azoxystrobin and HGCA Z, applied to the soil or to the foliage, on incidence and severity of take-all on the roots of wheat, measured at anthesis, grown in naturally-infested soil in Experiment 2.3¹.

Fungicide	Application to		
	Soil	Foliage	Mean
% plants with take-all			
None	+1.20(91.7)		+1.20 (91.7)
Azoxystrobin	+1.17(91.2)	+1.13 (90.5)	+1.15 (90.9)
HGCA Z	+1.20(91.7)	+1.13(90.5)	+1.16(91.1)
SED ² (83DF)	1	0.047	
	2	0.053	
Probability ³	0.69		0.69
% plants with moderate or severe take-all			
None	-0.14 (43.3)		-0.14 (43.3)
Azoxystrobin	-0.55 (25.1)	-0.25 (37.9)	-0.40 (31.2)
HGCA Z	+ 0.03 (51.6)	-0.56 (24.7)	-0.26 (37.2)
SED ² (83DF)	1	0.223	
	2	0.250	
Probability ³	0.006		0.40
Take-all index			
None	47.5		47.5
Azoxystrobin	42.2	45.8	44.0
HGCA Z	50.8	41.7	46.3
SED ² (83DF)	1	3.59	
	2	4.02	
Probability ³	0.01		0.38

¹Data (except take-all indices) are logit transform values with, in parentheses, the corresponding percentage values obtained by back transformation.

²SED 1 is for comparisons between the fungicide treatments, and SED 2 for comparing any of these with none.

³The probabilities that differences between the fungicides, excluding none, and their interactions with sites of application, occurred by chance.

DISCUSSION

The results from the field experiments described in this report, together with those previously published (Jenkyn *et al.*, 2000), provide convincing evidence that azoxystrobin applied as a foliar spray to winter wheat can give useful decreases in the severity of take-all on the roots. However, the magnitude (and significance) of the effects differed between experiments. Among the six experiments reported here, decreases in severity (plants with moderate or severe symptoms) ranged from *c* 20% (where azoxystrobin was applied twice in Experiment 1.4; not significant) to more than 80% (where azoxystrobin was applied once, at the beginning of stem extension, in Experiment 1.2). In the previously-reported work, the effects of azoxystrobin on take-all differed between cultivars but not consistently.

Effects on take-all of picoxystrobin (a more-recently introduced strobilurin fungicide than azoxystrobin, which was tested in five of the six experiments) were small and not significant. Much less information concerning their effects on take-all has been obtained for other fungicides from this group of compounds. However, results from the one field experiment (Experiment 1.5) that tested all six compounds that were available at the time, indicated that only one other (an experimental compound coded HGCA Z) has effects that are comparable to azoxystrobin. Results obtained from a pot experiment (Experiment 2.1) that compared the same fungicides (but applied to the soil) showed broadly similar effects of both azoxystrobin and HGCA Z. However, results from other pot experiments, which tested only these two compounds, were equivocal and so further work is needed to confirm the effects on take-all of HGCA Z. Apart from azoxystrobin and HGCA Z, one other compound (pyraclostrobin) had small effects on the severity of take-all in the field experiment (Experiment 1.5), and when applied at the larger of the two rates tested in the pot experiment (Experiment 2.1) but neither was significant. These effects were very modest compared to those of azoxystrobin (and HGCA Z) and, even if real, are unlikely to have any potential significance in the management of take-all.

In contrast, the effects of azoxystrobin (and, potentially, HGCA Z) on take-all can be comparable to those of the, relatively new, seed treatment fungicide fluquinconazole (Jenkyn, Gutteridge & Bateman, 2001), which is currently marketed for the control of this disease. Yield results from the Zeneca (Syngenta) experiments (Experiments 1.1 - 1.4) were not provided but in Experiment 1.5 the application of azoxystrobin and HGCA Z (at the beginning of stem extension and at flag leaf emergence) was associated with a 10% increase in grain yield compared to the application of related compounds or none.

The potential value of azoxystrobin in the practical management of take-all is, however, still uncertain because of remaining gaps in knowledge and understanding of its effects. The fact that these effects are somewhat variable would be less of a problem if they could be explained and especially if they could be

predicted. This may always be difficult but to make any progress at all is likely to depend on a better understanding of the mechanism by which azoxystrobin applied to the leaves decreases take-all on the roots. Unfortunately, the pot experiments that were intended to address this question (and which are considered in greater detail below) provided relatively little useful information.

Variability in the effects of azoxystrobin on take-all could also be of relatively less, or even no, importance if the use of the fungicide at a time that is appropriate for take-all control could be justified by the effects that it has on other diseases. As it has a (relatively) broad spectrum of activity, azoxystrobin is already applied widely to winter wheat in the UK. It is, however, most effective against the foliar diseases and much, but not all, is usually applied at later growth stages (e.g. GS 39, Jason Tatnell, personal communication) than those at which it has mostly been tested and been shown to be effective against take-all (GS 31-32). Although few data on the effects of later-applied sprays on take-all are available, there was a tendency in Experiment 1.3 for the severity of the disease to be decreased by a single spray at about early booting. However, there was a much larger (and significant) effect in this experiment where two sprays were applied, including one at the beginning of stem extension. Most of the experiments testing the effects of azoxystrobin on take-all have also used the full (250 g ai/ha) rate whereas many farmers use much less. Further work is needed to test the effects of applying azoxystrobin at different rates and at different growth stages.

From the evidence accumulated to date, there is no doubt that azoxystrobin can decrease take-all on the roots of winter wheat but the explanation for this has not been established beyond doubt. Although the effects are somewhat variable, they are surprisingly consistent, and sometimes surprisingly large, for a fungicide that is applied to the leaves. This is emphasised by the contrast between these results and those from previous experiments at Rothamsted, which showed that fungicides from the benzimidazole and SBI groups of compounds had effects on take-all only when applied to the soil at large rates of active ingredient and in large volumes of water (e.g. Bateman, 1989).

If azoxystrobin has a direct fungicidal effect, the compound, or fungicidally-active breakdown product(s), must be transported from the leaves to the roots either systemically through the plant or through the soil. Alternatively, take-all control could be an indirect effect of changes in the host.

Basipetal systemic movement of azoxystrobin itself can probably be discounted because it does not have the appropriate physicochemical properties to move in the phloem (Bromilow, Chamberlain & Evans, 1990; Godwin, Bartlett & Heaney, 1999). Structurally, the fungicide is an ester and one breakdown pathway in plants is hydrolysis to the free acid (Joseph, 1999). Theoretically, the acid has appropriate physicochemical properties to be translocated via phloem but there is no evidence that it is fungicidal (J.R. Godwin, personal communication). Other minor acid metabolites (Roberts & Hutson, 1999) may also be phloem mobile but it is improbable that they are fungitoxic. Enhanced resistance to take-all as an indirect consequence of the

effects that strobilurin fungicides can have on the physiology of wheat (e.g. Gerhard, Habermeyer & Zinkernagel, 1999) is a possibility. It is, however, considered to be an unlikely explanation because, with only one exception, compounds related to azoxystrobin that are known or can be expected to have similar physiological effects had no significant effect on take-all.

On current evidence, movement of azoxystrobin to the roots through soil is, therefore, considered to be the most likely explanation for the effects that we describe. It is, nevertheless, difficult to reconcile this with some of the evidence and assumptions concerning the effects and behaviour of azoxystrobin in crops. Because it is moderately lipophilic, azoxystrobin should be sorbed to soil and, therefore, relatively immobile, particularly under (UK) summer conditions. However, it is the least lipophilic of the currently-available strobilurin fungicides (Table 11) and, therefore, more likely to move through soil than related compounds. It also has a much longer half life in soil than related compounds apart from picoxystrobin (Table 11). Results from the three pot experiments described in this report also confirm that azoxystrobin is effective against take-all when applied to the soil but it had no significant effect when applied only to the foliage, using a paint brush, in Experiment 2.3.

Table 11. Octanol/water partition coefficients (Log K_{ow}) and half lives in soil of strobilurin fungicides (Nicholls, 1994)

Fungicide	log K_{ow}	Mean half life in soil (range) in days
Azoxystrobin	2.5	>28 (7-40)
Kresoxim-methyl	3.4	1 (0.5 - 3)
Picoxystrobin	3.6	28 (7 - 40)
Pyraclostrobin	4.0	12 (2 - 37)
Trifloxystrobin	4.5	6 (4 - 10)

However, even if azoxystrobin has the potential to be active in, and to move through, soil, it is presumably necessary for significant (but, at present, unknown) amounts of active ingredient to reach the soil if there are to be biologically significant effects on take-all. Some fungicide will be deposited on the soil during spraying but the amount (proportion) will depend on the density of the crop which will be determined by a number of factors including crop architecture, the growing conditions previously experienced by the crop and its growth stage. Differences in the densities of crops at the same growth stage can be considerable (Becker *et al.*, 1999), and it is conceivable that this could explain some of the differences in effects of azoxystrobin in different experiments, and on different cultivars in the experiments reported by Jenkyn *et al.* (2000). Nevertheless, average ground cover in winter wheat crops at the early stem extension stage is about 60%, and sometimes much greater (Becker *et al.*, 1999) implying that less than half of the spray would

normally be deposited on the soil. This may be augmented by fungicide washed from the leaves to the soil by rain falling after sprays have been applied but the formulations used for foliar fungicides are designed to avoid this, and most are considered to be rain-fast within a few hours. The implication is that azoxystrobin is intrinsically very active against the take-all fungus.

To be effective against take-all it seems likely that fungicide deposited on the soil surface must then be distributed through the soil by rain (or irrigation). Circumstantial evidence for this is, perhaps, provided by Experiment 1.6 in which there were no effects on take-all of applying sprays to the foliage of winter wheat sown at different row spacings or of applying them directly to the soil, which must have resulted in different, and in some plots relatively large, deposits on the soil surface. In the pot experiments there were no consistent effects of top vs bottom watering but redistribution is probably less important in the restricted confines of a pot than in the field. Also, movement of water as a consequence of its uptake into the soil by capillary action and subsequent absorption by roots probably ensured that there was some downward redistribution of fungicide even where pots were watered from below.

How far down the soil profile fungicide needs to be moved in the field is uncertain but our evidence for effects of azoxystrobin on take-all is based on roots sampled to a depth of 10-15 cm. These samples are typically taken in late June or early July; some two - three months after the application of sprays at the beginning of stem extension. However, if the fungicide is to have significant effects on take-all it probably needs to be moved from the surface of the soil within a much shorter period, perhaps a month or less. It seems likely that, in many years and given the known physicochemical properties of azoxystrobin, insufficient rain will fall within this sort of period to move significant amounts of the fungicide to this sort of depth. Some fungicide may be transported to these, or greater, depths down cracks and large pores but probably only a relatively small proportion. Even if there is significant rain, it will probably be much less effective in moving the fungicide if it falls on dry soil than if it falls on soil that is already at field capacity (Allan Walker, personal communication).

Despite the assumed importance of rainfall and soil moisture content in determining the movement of azoxystrobin through soil, the association between them and the effects that the fungicide had on take-all in different experiments is not close. In Experiment 1.6 in 2001, where there was little effect on take-all even where the fungicide was applied to the soil, the potential soil moisture deficit (Psm_d) on the day that the early spray was applied (11 May) was 19.2 mm (Table 12). There was significant rain on the 4th - 6th days after spraying (33.6 mm) and this was sufficient to eliminate the soil moisture deficit. Rainfall in the following three weeks was negligible. Psm_d was very similar when sprays were applied on 9 April to the 1999 experiment described by Jenkyn *et al.* (2000) (Expt 2; Table 12), and total rainfall in the following two weeks (45.8 mm) was broadly similar to the amount that fell in the first week after spraying in 2001. However, in this experiment, there was a significant effect on take-all. In the other two experiments listed in

Table 12 (Experiment 1 of Jenkyn *et al.* (2000) and Experiment 1.5 in this report), Psmd was small and similar when sprays were applied but rainfall in the following 2-3 weeks was very different. Despite this there were significant effects on take-all in both experiments although the possibility that the second spray applied to Experiment 1.5 on 20 May might have contributed to this cannot be discounted. Experiment 1.5 was also sited on the Woburn, not Rothamsted, Experimental Farm but on a soil which, if anything, is heavier than the clay loam soils at Rothamsted. It should be emphasised, however, that although the (early) sprays were applied at similar growth stages in all four experiments, they covered a wide range of calendar dates. It may be significant that the experiment in 2001 was sprayed much later than the previous three.

Table 12. Potential soil moisture deficits (Psmd) when azoxystrobin sprays were applied (at the beginning of stem extension) to four different experiments, rainfall in each of the following four weeks and measured reductions in the severity of take-all

Experiment ¹ (Date sprayed)	Psmd when sprayed	Rain (mm) in 4 weeks after spraying				% reduction in take-all	
		1 st week	2 nd week	3 rd week	4 th week	Mod + Sev	Sev
Expt1 (7 April 1998)	0.0	38.1	23.7	15.2	1.9	19.4	63.2
Expt2 (9 April 1999)	18.9 ³	19.5	26.3	1.2	4.6	2.9 (NS)	28.6
Expt 1.5 ² (27 April 2000)	2.3 ⁴	7.0	3.2	11.2	28.2	56.6	- ⁶
Expt 1.6 (11 May 2001)	19.2 ⁵	35.2	0.0	0.1	0.8	-23.7 ⁷ (NS)	- ⁶

¹ Experiments 1 and 2 were at Rothamsted (see Jenkyn *et al.* (2000) for further details). Experiments 1.5 and 1.6 (described in this report) were at Woburn and Rothamsted, respectively.

² In this experiment, plots were sprayed a second time, on 20 May.

³ Psmd eliminated on 20 April, 11 days after spraying.

⁴ Psmd eliminated on 28 April, 1 day after spraying.

⁵ Psmd eliminated on 17 May, 6 days after spraying.

⁶ Little severe take-all.

⁷ Effect of foliar sprays applied in spring to plots sown at the narrow row spacing only.

This discussion has focussed on the effects of azoxystrobin because that is the compound for which most data are currently available but HGCA Z had very similar effects in the one field experiment in which it was tested (Experiment 1.5) and in the first of the pot experiments (Experiment 2.1). In the other two pot experiments there were, however, apparent differences in the behaviour and effects of the two fungicides. In both experiments, take-all was significantly decreased by azoxystrobin applied to the soil but not by HGCA Z. In contrast, there was evidence from Experiment 2.3 that take-all was decreased by HGCA Z applied to

the foliage but this result must be treated with caution especially as the fungicide had no effect when applied to the soil in the same experiment. In Experiment 2.2 there were a number of significant interactions involving watering regimes but the apparent effects of top *vs* bottom watering were inconsistent and probably spurious. Different effects of large *vs* small rates of application for the two fungicides in Experiment 2.2 were, however, more consistent being very similar for plants sampled after 4 weeks or after 8 weeks. These results, which indicated that azoxystrobin was more effective at the smaller than at the larger of the two rates tested (possibly indicating slight phytotoxicity at the larger rate) were not dissimilar to the effects of azoxystrobin in Experiment 2.1. In the latter experiment, HGCA Z was, in contrast, more effective in decreasing take-all at the larger than at the smaller rate. The average effects of HGCA Z in Experiment 2.2 were small and not significant but after 8 weeks it, similarly, seemed to decrease the severity of take-all at the large rate but not at the small. Further work is, therefore, needed to confirm the effects on take-all of HGCA Z. By comparison with azoxystrobin this may help to explain the behaviour and effects of both.

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REFERENCES

- Bateman, G.L. (1989). Progress in research on the control of take-all in cereals with fungicides: a review. *Crop Protection* **8**, 75-81.
- Beale, R.E., Phillion, D.P., Headrick, J.M., O'Reilly, P. & Cox, J. (1998). Mon 65500: A unique fungicide for the control of take-all in wheat. *Proceedings of the 1998 Brighton Conference - Pests & Diseases* **2**, 343-350.
- Becker, F.A., Klein, A.W., Winkler, R., Jung, B., Bleiholder, H. & Schmider, F. (1999). The degree of ground coverage by arable crops as a help in estimating the amount of spray solution intercepted by the plants. *Nachrichtenblatt für den Deutschen Pflanzenschutzdienst* **51**, 237-242.
- Bromilow, R.H., Chamberlain, K. & Evans, A.A. (1990). Physicochemical aspects of phloem translocation of herbicides. *Weed Science* **38**, 305-314.
- Gerhard, M., Habermeyer, J. & Zinkernagel, V. (1999). The impact of strobilurins on plant vitality on winter wheat under field conditions. In *Modern Fungicides and Antifungal Compounds II*, pp. 197-208. Eds. H. Lyr, P.E. Russell, H.-W. Dehne and H.D.J. Sisler. Andover: Intercept Ltd.
- Godwin, J.R., Bartlett, D.W. & Heaney, S.P. (1999). Azoxystrobin: implications of biochemical mode of action. Pharmacokinetics and resistance management for spray programmes against septoria diseases of wheat. In *Septoria on Cereals: a Study of Pathosystems*, pp. 299-315. Eds. J.A. Lucas, P. Bowyer and H.M. Anderson. Wallingford: CAB International.
- Hornby, D., Bateman, G.L., Gutteridge, R.J., Lucas, P., Osbourn, A.E., Ward E. & Yarham, D.J. (1998). *Take-all Disease of Cereals: A Regional Perspective*. Wallingford: CAB International.
- Jenkyn, J.F., Bateman, G.L., Gutteridge, R.J. & Edwards, S.G. (2000). Effects of foliar sprays of azoxystrobin on take-all in wheat. *Annals of Applied Biology* **137**, 99-106.
- Jenkyn, J.F., Gutteridge, R.J. & Bateman, G.L. (2001). Effects of fluquinconazole seed treatment on take-all and yield of winter wheat, and its exploitation in cropping systems. *BCPC Symposium Proceedings No. 76: Seed Treatment: Challenges and Opportunities*, pp. 91-98.

- Joseph, R.S.I. (1999). Metabolism of azoxystrobin in plants and animals. In *Pesticide Chemistry and Bioscience: The Food-Environment Challenge*, pp. 265-278. Eds G.T. Brooks and T.R. Roberts. Cambridge: The Royal Society of Chemistry.
- Löchel, A.M., Wenz, M., Russell, P.E., Buschhaus, H., Evans, P.H., Cross, S., Puhl, T. & Bardsley, E. (1998). Root protection using fluquinconazole: a new approach to controlling cereal take-all. *Proceedings of the 1998 Brighton Conference - Pests & Diseases* **1**, 89-96.
- Nicholls, P.H. (1994). 'Physicochemical evaluation: the environment' an expert system for pesticide preregistration assessment. *Proceedings of the 1994 Brighton Crop Protection Conference - Pests and Diseases* **3**, 1337-1342.
- Prew, R.D., Ashby, J.E., Bacon, E.T.G., Christian, D.G., Gutteridge, R.J., Jenkyn, J.F., Powell, W. & Todd, A.D. (1995). Effects of incorporating or burning straw, and of different cultivation systems, on winter wheat grown on two soil types, 1985-91. *Journal of Agricultural Science, Cambridge* **124**, 173-194.
- Roberts, T.R. & Hutson, D.H. (Editors in Chief) (1999). *Metabolic Pathways of Agrochemicals. Part 2: Insecticides and Fungicides*. Cambridge: The Royal Society of Chemistry.
- Soika, M.D. & Sanders, P.L. (1996). Evaluation of fungicides for control of take-all patch, 1995. *Fungicide and Nematicide Tests* **51**, 364.
- Spink, J.H., Wade, A.P., Paveley, N.D., Griffin, J.M., Scott, R.K. & Foulkes, M.J. (1998). The effects of a novel seed treatment, Mon 65500, on take-all severity and crop growth in winter wheat. *Proceedings of the 1998 Brighton Conference - Pests & Diseases* **3**, 913-920.
- Wenz, M., Russell, P.E., Löchel, A.M., Buschhaus, H., Evans, P.H., Bardsley, E., Petit, F. & Puhl, T. (1998). Seed treatment with fluquinconazole for control of cereal take-all, foliar and seed-borne diseases. *Proceedings of the 1998 Brighton Conference - Pests & Diseases* **3**, 907-912.
- Zadoks, J.C., Chang, T.T. & Konzak, C.F. (1974). A decimal code for the growth stages of cereals. *Weed Research* **14**, 415-421.