

# Strobilurin fungicides induce changes in photosynthetic gas exchange that do not improve water use efficiency of plants grown under conditions of water stress

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**Abstract:** The effects of five strobilurin (beta-methoxyacrylate) fungicides and one triazole fungicide on the physiological parameters of well-watered or water-stressed wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and soya (*Glycine max* Merr.) plants were compared. Water use efficiency (WUE) (the ratio of rate of transpiration,  $E$ , to net rate of photosynthesis,  $A_n$ ) of well-watered wheat plants was improved slightly by strobilurin fungicides, but was reduced in water-stressed plants, so there is limited scope for using strobilurins to improve the water status of crops grown under conditions of drought. The different strobilurin fungicides had similar effects on plant physiology but differed in persistence and potency. When applied to whole plants using a spray gun, they reduced the conductance of water through the epidermis (stomatal and cuticular transpiration),  $g_{sw}$ , of leaves. Concomitantly, leaves of treated plants had a lower rate of transpiration,  $E$ , a lower intercellular carbon dioxide concentration,  $c_i$ , and a lower net rate of photosynthesis,  $A_n$ , compared with leaves of control plants or plants treated with the triazole. The mechanism for the photosynthetic effects is not known, but it is hypothesised that they are caused either by strobilurin fungicides acting directly on ATP production in guard cell mitochondria or by stomata responding to strobilurin-induced changes in mesophyll photosynthesis. The latter may be important since, for leaves of soya plants, the chlorophyll fluorescence parameter  $F_v/F_m$  (an indication of the potential quantum efficiency of PSII photochemistry) was reduced by strobilurin fungicides. It is likely that the response of stomata to strobilurin fungicides is complex, and further research is required to elucidate the different biochemical pathways involved.

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**Keywords:** strobilurin fungicides; wheat; photosynthesis; stomata; water use efficiency; chlorophyll fluorescence

## 1 INTRODUCTION

Strobilurin fungicides are beta-methoxyacrylate compounds that inhibit respiration in fungi by binding to the Q<sub>o</sub> site of the cytochrome  $bc_1$  complex located in the inner mitochondrial membrane.<sup>1–3</sup> This blocks electron transport and so reduces ATP synthesis. Strobilurin fungicides inhibit respiration in cytochrome  $bc_1$  isolated from yeast, fly, rat and corn<sup>4</sup> and wheat and beef heart.<sup>3</sup> They also inhibit respiration in intact wheat plants<sup>5</sup> and in spinach (*Spinacia oleracea* L.) leaf discs.<sup>6</sup>

Strobilurin fungicides induce physiological changes in wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) plants, including delayed senescence,<sup>7–10</sup> altered amounts of phytohormones,<sup>9</sup> increased activity of antioxidative enzymes<sup>8,11</sup> and increased activity of nitrate reductase in the dark.<sup>5,6</sup> Not all of these physiological effects are unique to strobilurin fungicides. For example epoxiconazole, a triazole fungicide that inhibits sterol biosynthesis, also delays senescence and increases the activity of antioxidative enzymes.<sup>8,11</sup>

Application to wheat plants of fungicidal preparations containing the strobilurin kresoxim-methyl reduces stomatal aperture<sup>9</sup> yet increases the net rate of photosynthesis,  $A_n$ , of treated leaves.<sup>5,9</sup> In general, the reverse should be true, since there is a well-established positive relationship between  $A_n$  and stomatal transpiration,  $g_{sw}$ ,<sup>12–14</sup> as stomatal opening increases  $A_n$  by increasing diffusive transport of carbon dioxide.<sup>15</sup> Indeed, a representative methoxyacrylate reduced oxygen evolution from treated barley leaves by more than 50% under conditions of saturating light.<sup>16</sup> Since stomatal aperture also regulates water loss through transpiration,  $E$ , by reducing loss of water through the stomata, it has been suggested that strobilurin fungicides may improve the water status and stress management of plants under conditions of water deficit.<sup>6,9</sup>

The aim of the work reported here was to assess the potential for using strobilurin fungicides to improve the water status of plants grown under conditions of either light or severe water stress. In the first such comparison, the authors tested five different commercially

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available strobilurin fungicides that each have the same mode of fungicidal action, inhibition of mitochondrial respiration, but different biokinetic properties, for example lipophilicity and potential for translaminal movement.<sup>17</sup> A dose–response experiment was used to ascertain whether the photosynthetic effects likely to alter the water status of wheat plants are related to the amount of strobilurin applied. Two strobilurin fungicides were compared: picoxystrobin, which is a xylem systemic fungicide also capable of significant translaminal movement,<sup>1</sup> and pyraclostrobin, which, in contrast, is not xylem systemic and exhibits only limited translaminal movement.<sup>1</sup> Selected strobilurin fungicides with different biokinetic properties were also applied to barley and soya plants to assess whether their photosynthetic effects are broadly similar in cereals and broad-leaved plants. In addition to measuring gas exchange, the authors chose soya plants as a model system for measuring chlorophyll fluorescence. Specifically, the aim was to test the following hypotheses:

1. All strobilurin fungicides have the same effect on the photosynthetic parameters of wheat, barley and soya. Since strobilurin fungicides have different biokinetic properties, the magnitude and duration of the effect depend on the specific strobilurin.
2. The magnitude of the effects of strobilurin fungicides on the photosynthetic parameters of wheat increases with dose.
3. Strobilurin fungicides improve the water use efficiency (WUE) (the ratio of  $E$  to  $A_n$ ) of treated leaves by reducing the rate of loss of water through the stomata more than the rate of net photosynthesis.

## 2 MATERIALS AND METHODS

### 2.1 Plant growth conditions

Wheat (*Triticum aestivum* L. cv. Consort) and barley (*Hordeum vulgare* L. cv. Golden Promise) plants were grown in a heated ( $\geq 5^\circ\text{C}$ ) glasshouse with natural lighting. Seeds were soaked in aerated distilled water for 24 h prior to sowing four at 2 cm depth in 1 dm<sup>3</sup> plastic pots containing 800 g moist compost (John Innes No. 3). After 7 days, pots were assigned randomly to treatment and thinned to two seedlings per pot.

Seeds of soya [*Glycine max* L. (Merr.) cv. Amsoy] were soaked in aerated distilled water for 24 h, rinsed in fresh distilled water and then germinated on moist filter paper in a propagator. After 5 days the seedlings were transferred to hydroponic culture. Each seedling was grown in a 1 dm<sup>3</sup> beaker containing 800 cm<sup>3</sup> half-strength Long Ashton nutrient solution (full strength: KNO<sub>3</sub> 4, Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O 4, NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O 1.33, MgSO<sub>4</sub>·7H<sub>2</sub>O 1.5, FeEDTA Na 0.1, MnSO<sub>4</sub>·4H<sub>2</sub>O 0.01, CuSO<sub>4</sub>·5H<sub>2</sub>O 0.001, ZnSO<sub>4</sub>·7H<sub>2</sub>O 0.001, H<sub>3</sub>BO<sub>3</sub> 0.05, Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O 0.004, NaCl 0.1, Na<sub>2</sub>SiO<sub>3</sub>·5H<sub>2</sub>O 0.05 mM). Beakers

were wrapped in metal foil to prevent growth of algae, and the nutrient solution was changed twice weekly and aerated through aquarium airstones at a flowrate of 0.1 dm<sup>3</sup> min<sup>-1</sup>. Plants were grown in a controlled environment cabinet (Sanyo Gallenkamp, Loughborough, Leics) at 80% RH, 25 °C and ambient carbon dioxide concentration (approximately 380 ppm). Illumination was by sodium halide HQI lights supplemented with tungsten filament lamps giving a photosynthetic photon flux density (PPFD) of approximately 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at plant height during a 16 h photoperiod.

### 2.2 Light and severe water stress

Five strobilurin fungicides were evaluated: picoxystrobin, pyraclostrobin, azoxystrobin, kresoxim-methyl and trifloxystrobin. The effects of these strobilurin fungicides on well-watered or water-stressed wheat plants were compared in two experiments. In the first, all five strobilurins were compared with a triazole fungicide, epoxiconazole (Opus<sup>®</sup>; BASF, Limburgerhof), on plants grown with no or light water stress (0 to  $-0.1$  MPa or  $-0.7$  to  $-0.8$  MPa). In the second, four of the strobilurin fungicides, picoxystrobin, pyraclostrobin, azoxystrobin and trifloxystrobin, were compared with plants grown under conditions of no or severe ( $-1.5$  MPa) water stress. A drying curve for John Innes No. 3 compost was constructed by measuring the water potential of the compost over a range of moisture contents using a psychrometer (WP4 Dewpoint Potentiometer; Decagon Devices, Inc., Pullman, WA). Compost in all pots was maintained at  $-0.10 \pm 0.05$  MPa until 7 days after sowing, by weighing and watering twice weekly. Before strobilurin fungicides were applied, the moisture content of the compost in pots assigned to the water stress treatment was allowed to decline until the target soil water potential was attained (either  $-0.70$  to  $-0.80$  or  $-1.50$  MPa). Thereafter, the soil water potential was kept constant by weighing and watering daily. In the first drought stress experiment, three replicate plants were sampled from each treatment on the first, third and seventh days after spraying (DAS). The same sampling intervals were used in the second drought stress experiment, but four replicate plants were sampled from each treatment.

### 2.3 Application of fungicides

Prior to treatment with strobilurins, plants in all experiments were kept visually free of disease by applying quinoxifen (Fortress<sup>®</sup>; 10 mg AI L<sup>-1</sup>) with a hand-held spray gun 7 days after the seeds were sown. Treatment fungicides were applied to the plants in each pot individually, 1 day after full expansion of the second emergent leaf on the main stem. Wheat plants in the severe water stress treatment grew more slowly than well-watered plants and so were sprayed 2 days after the well-watered plants so that plants in each treatment were at the same stage of development. Strobilurin fungicide solutions

were prepared on the morning of spraying in distilled water + acetone (50 + 50 by volume) containing  $1 \text{ g L}^{-1}$  Tween-20 and were applied at full field rate ( $1.25 \text{ g AI dm}^{-3}$ ) to soya and at half field rate to wheat and barley plants. Control plants received distilled water only, and, for the 'blank' treatment, plants were sprayed with distilled water + acetone (50 + 50 by volume) containing  $1 \text{ g L}^{-1}$  Tween-20. For the dose-response experiment, four replicate wheat plants were treated with either picoxystrobin or pyraclostrobin at concentrations of 0, 0.25, 0.5, 1 or 2 mM. Plants were placed on a turntable (3 rpm) and sprayed for 20 s (after which time there was visible deposition of fungicide upon the second fully expanded leaf) using a deVilbiss spray gun at 10 psi pressure. Fungicides were applied in random order, and the spray gun was rinsed with acetone and then with distilled water and dried between each application.

#### 2.4 Measurement of gas exchange

On the day of sampling, wheat or barley plants were transferred to a custom-built controlled growth room ( $20^\circ\text{C}$ , RH 76%) under PPFd of  $520\text{--}600 \mu\text{mol m}^{-2} \text{ s}^{-1}$  at plant height. Rates of carbon dioxide uptake and water vapour loss were determined for the mid-section of the second fully expanded leaf on the main stem of one plant in each pot using an infrared gas analyser (IRGA) with a narrow-leaf cuvette (CIRAS-1; PP Systems, Hitchin, Herts). For each leaf, four measurements were taken at 20 s intervals, 14 min after placing the leaf in the cuvette.

#### 2.5 Light response

The response of photosynthetic parameters of the second fully expanded leaf of wheat to increasing light was determined. Wheat plants were treated either with a blank solution (acetone + distilled water, 50 + 50 by volume) or with picoxystrobin at half field rate ( $0.625 \text{ g dm}^{-3}$ ) 2 days after full expansion of the second emergent leaf on the main stem. At 1 DAS, plants were transferred to a controlled environment growth cabinet, as described in Section 2.1. During a 16 h photoperiod, PPFd at plant height was increased from 0 to approximately  $950 \mu\text{mol m}^{-2} \text{ s}^{-1}$  by increasing the power supplied to the lamps. Photosynthetic parameters were recorded every 5 minutes using a CIRAS-1 IRGA.

#### 2.6 Chlorophyll fluorescence

Picoxystrobin and pyraclostrobin were applied 5 days after full expansion of the first emergent trifoliate leaf. At 2 DAS, photosynthetic parameters were measured using a CIRAS-1 with a broad-leaf cuvette. Following measurement of gas exchange, leaves were dark adapted for 30 min to allow complete reoxidation of photosystem II (PSII) electron-acceptor molecules, and fluorescence was then measured using a Hansatech fluorescence monitoring system (Hansatech Instruments Ltd, King's Lynn, Norfolk).

Minimal chlorophyll fluorescence,  $F_0$ , was measured under a modulating beam only, and then the leaflets received a saturating pulse (determined on spare plants) of actinic light to measure maximal chlorophyll fluorescence,  $F_m$ . Variable fluorescence,  $F_v$ , was calculated as  $F_m$  minus  $F_0$ , and  $F_v/F_m$  was calculated as an indicator of the maximum quantum efficiency of PSII.<sup>18,19</sup>

#### 2.7 Data analysis

Data were tested for normality using a Kolmogorov-Smirnov test, and treatment means were compared using one-way analysis of variance (ANOVA) with *post hoc* tests of least significant difference (LSD) performed using SPSS 11.0 (SPSS Inc., Chicago, IL). Correlation coefficients,  $r$ , and significance of fit,  $p$ , of different equations to the data were computed by least-squares regression analysis in Sigmaplot v. 8 (SPSS Inc., Chicago, IL). Net rates of photosynthesis,  $A_n$ , transpiration,  $E$ , and stomatal conductance,  $g_{sw}$ , and intercellular carbon dioxide concentration,  $c_i$ , were determined using CIRAS-1 software based on the equations of von Caemmerer and Farquhar.<sup>20</sup> The instantaneous water use efficiency (WUE) of the leaves was calculated as the ratio of  $E$  to  $A_n$  [ $\text{mol H}_2\text{O}(\text{mol CO}_2)^{-1}$ ] as described by Lawlor.<sup>21</sup>

### 3 RESULTS

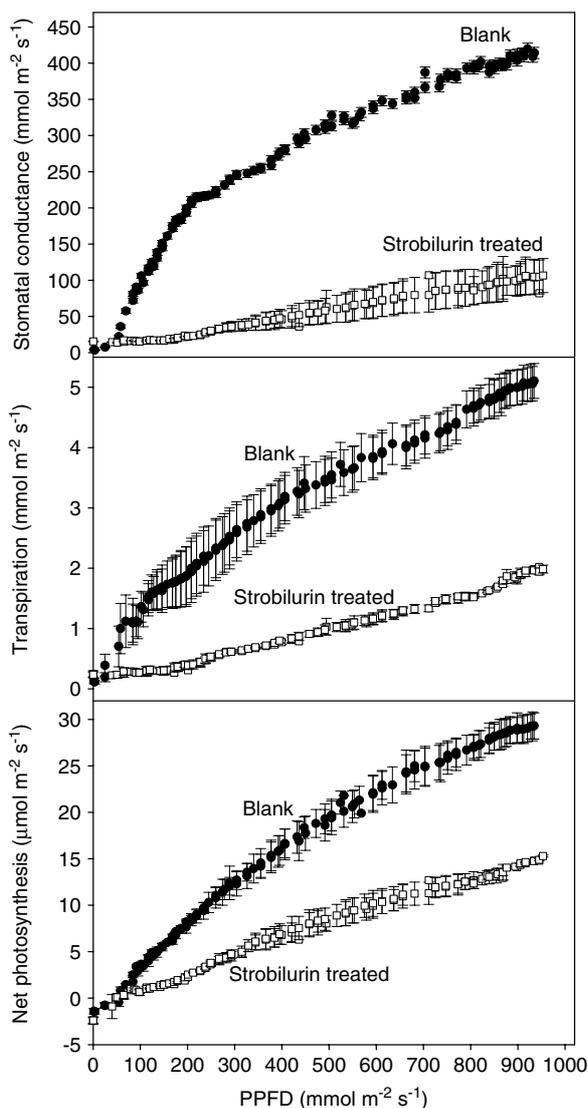
In all experiments, strobilurin fungicides reduced the rate of conductance of water through stomata,  $g_{sw}$ , the rates of transpiration,  $E$ , and net photosynthesis,  $A_n$ , and the intercellular carbon dioxide concentration,  $c_i$ , of treated leaves relative to leaves treated with blank solvent (distilled water + acetone, 50 + 50 by volume) or a triazole fungicide (epoxiconazole). This was true for wheat (Figs 1–6), barley (Fig. 7) and soya (Fig. 8) plants.

#### 3.1 Light response

Photosynthetic parameters ( $g_{sw}$ ,  $A_n$  and  $E$ ) of both strobilurin-treated plants and plants treated with blank solvent increased with photosynthetic photon flux density (PPFD) (Fig. 1), while  $c_i$  decreased with increasing  $A_n$  (Fig. 2). Rates  $g_{sw}$ ,  $A_n$  and  $E$  at maximum light intensity were reduced by approximately 76, 48 and 56% respectively by picoxystrobin. The relationships between PPFd and  $g_{sw}$ ,  $A_n$  and  $E$  were described by linear equations, but were often described better using a single rectangular hyperbola (Table 1). The  $y_0$  values indicate that the mean rate of respiration of leaves following application of picoxystrobin was reduced by approximately 60% relative to leaves treated with blank solvent.

#### 3.2 Dose response

Photosynthetic parameters ( $g_{sw}$ ,  $A_n$ ,  $E$  and  $c_i$ ) were reduced by treating leaves of wheat plants with either picoxystrobin or pyraclostrobin (Fig. 3). The

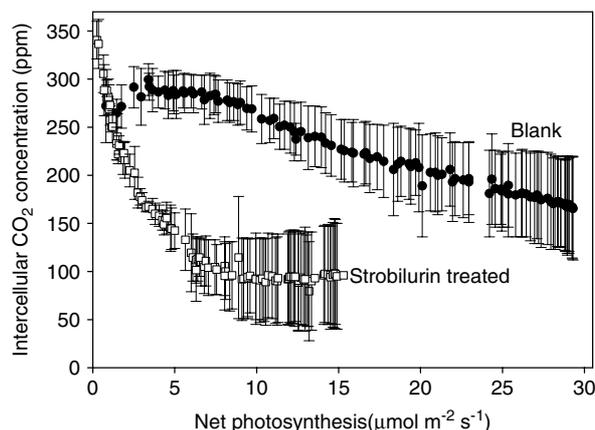


**Figure 1.** Response of the rate of stomatal conductance to water (top), rate of net transpiration (middle) and rate of net photosynthesis (bottom) of the mid-portion of the second fully expanded leaf on the main stem of wheat to increasing photosynthetic photon flux density (PPFD). Plants were measured 1 day after spraying with distilled water + acetone, 50 + 50 by volume (blank), or with half field rate (0.625 g dm<sup>-3</sup>) picoxystrobin in distilled water + acetone, 50 + 50 by volume (strobilurin treated). Data are mean ± SE, *n* = 3.

effects were related to the dose applied, up to approximately 0.5 mM. Increasing the concentration of either fungicide to above 0.5 mM did not result in further reductions in photosynthetic parameters. At each dose, picoxystrobin decreased  $g_{sw}$ ,  $A_n$  and  $E$  more than pyraclostrobin.

### 3.3 Water stress

Both light (−0.75 MPa) and severe (−1.5 MPa) water stress reduced the  $g_{sw}$  of the second emergent leaf on the main stem of wheat plants measured 1, 3 and 7 DAS, with concomitant reductions in  $A_n$ ,  $E$  and  $c_i$  of the same leaf. Applying strobilurin fungicides further reduced the rates  $g_{sw}$ ,  $A_n$  and  $E$  and  $c_i$  of leaves of treated plants relative to those of plants treated with distilled water (control). At the seventh day,



**Figure 2.** Relationship between the rate of net photosynthesis and the intercellular carbon dioxide concentration of the mid-portion of the second fully expanded leaf on the main stem of wheat with increasing photosynthetic photon flux density (PPFD). Plants were measured 1 day after spraying with distilled water + acetone, 50 + 50 by volume (blank), or half field rate (0.625 g dm<sup>-3</sup>) picoxystrobin in distilled water + acetone, 50 + 50 by volume (strobilurin treated). Data are mean (± SE), *n* = 3.

leaves from plants subjected to severe water stress and treated with the blank solvent (blank) had significantly ( $P < 0.05$ ) lower  $g_{sw}$  than leaves from control plants, but the effect of the blank solvent was not significant at the first and third days and was never significant for well-watered plants.

The relationship between  $A_n$  and  $g_{sw}$  of plants from all treatments in the severe water stress experiment could be described using a single equation. In Fig. 4 and Table 2 a single rectangular hyperbola has been used, where  $A_n = a \times g_{sw} / (b + g_{sw})$ .  $E$  is also well predicted by  $g_{sw}$  using the same rectangular hyperbola. The same model predicts the  $c_i$  of leaves from well-watered and water-stressed plants, but underestimates the  $c_i$  of leaves from plants that had been both subjected to water stress and treated with strobilurin fungicides (Fig. 4).

The strobilurin fungicides differed in potency and in persistence. Kresoxim-methyl had the smallest effect on photosynthetic parameters of leaves of wheat plants (Fig. 5), while picoxystrobin and pyraclostrobin often had the greatest effect (Figs 5 and 6). In both the light and severe water stress experiments the effects of strobilurin fungicides were still noticeable 7 DAS. Plants treated with pyraclostrobin recovered least, with  $g_{sw}$  depressed by approximately 70% at 7 DAS (Fig. 5).

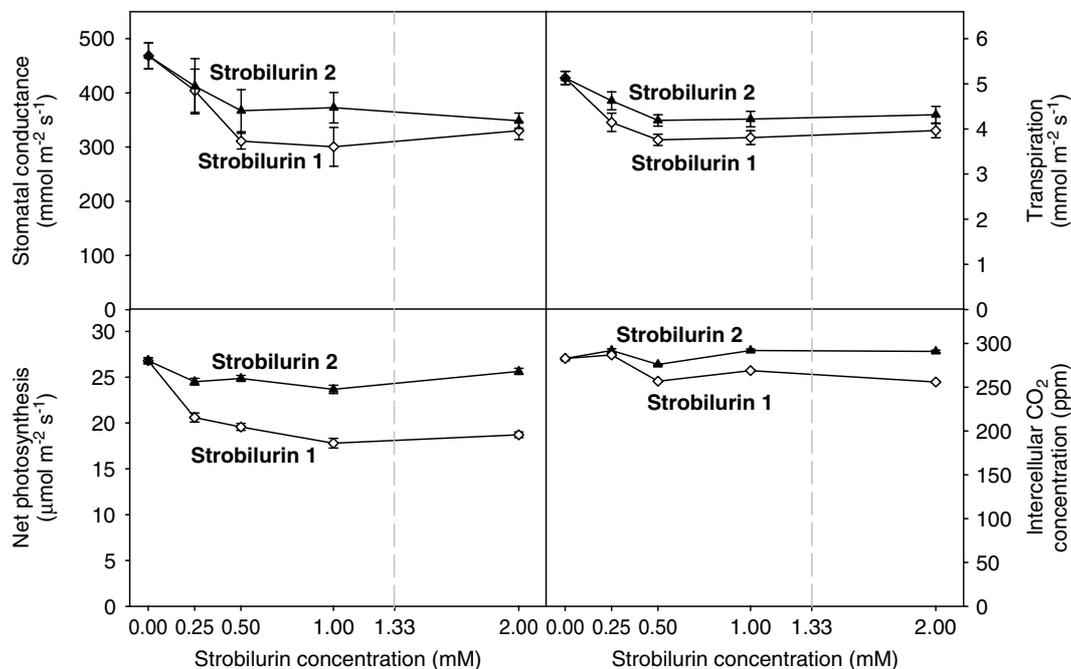
Pyraclostrobin, azoxystrobin and trifloxystrobin improved the WUE of leaves from well-watered plants by reducing the amount of water required to fix 1 mol of carbon dioxide (Table 3). Conversely, the WUE of leaves from severely water-stressed plants was reduced following treatment with these strobilurins, relative to the blank solution.

### 3.4 Chlorophyll fluorescence

Picoxystrobin and pyraclostrobin increased minimal fluorescence,  $F_0$ , and decreased the maximal fluorescence yield,  $F_m$ , of dark-adapted soya plants

**Table 1.** Parameters, significance and goodness of fit of either a linear equation ( $y = y_0 + a \times \text{PPFD}$ ) or a single rectangular hyperbola [ $y = (a \times \text{PPFD}) / (b + \text{PPFD})$ ] to the relationships between the rates of stomatal conductance to water,  $g_{sw}$ , net photosynthesis,  $A_n$ , and transpiration,  $E$ , with photosynthetic photon flux density (PPFD). Plants were measured 1 day after spraying with distilled water + acetone, 50 + 50 by volume (blank), or with half field rate ( $0.625 \text{ g dm}^{-3}$ ) picoxystrobin in distilled water + acetone, 50 + 50 by volume. Data are mean  $\pm$  SE,  $n = 112$

Variable	Treatment	$y_0$	$a$	$r$	$P$
$g_{sw}$	Blank	$1.73 \pm 2.6$	$0.58 \pm 6 \times 10^{-2}$	0.952	<0.001
	Strobilurin 1	$5.68 \pm 4.2$	$0.10 \pm 2 \times 10^{-2}$	0.973	<0.001
$E$	Blank	$0.14 \pm 0.1$	$6 \times 10^3 \pm 1 \times 10^{-3}$	0.967	<0.001
	Strobilurin 1	$0.11 \pm 0.1$	$2 \times 10^3 \pm 3 \times 10^{-4}$	0.971	<0.001
$A_n$	Blank	$-1.97 \pm 0.4$	$1.4 \times 10^{-2} \pm 4 \times 10^{-3}$	0.968	<0.001
	Strobilurin 1	$-0.80 \pm 0.1$	$1.7 \times 10^{-2} \pm 2 \times 10^{-3}$	0.981	<0.001
Variable	Treatment	$a$	$b$	$r$	$\rho$
$g_{sw}$	Blank	$621 \pm 9$	$479 \pm 15$	0.996	<0.001
	Strobilurin 1	$841 \pm 209$	$6.6 \times 10^3 \pm 1.8 \times 10^3$	0.989	<0.001
$E$	Blank	$9.0 \pm 0.2$	$688 \pm 24$	0.996	<0.001
	Strobilurin 1	$2.0 \times 10^4 \pm 9 \times 10^5$	$9.7 \times 10^6 \pm 5 \times 10^8$	0.993	<0.001
$A_n$	Blank	$97 \pm 5$	$2.7 \times 10^3 \pm 140$	0.996	<0.001
	Strobilurin 1	$4.5 \times 10^3 \pm 2 \times 10^4$	$2.8 \times 10^5 \pm 3 \times 10^5$	0.991	<0.001



**Figure 3.** Rate of stomatal conductance to water of the second fully expanded leaf on the main stem of wheat plants, measured 2 days after application of picoxystrobin (strobilurin 1) or pyraclostrobin (strobilurin 2) at 0, 0.25, 0.5, 1 and 2 mM in distilled water + acetone, 50 + 50 by volume. Dashed line denotes approximate half field rate (1.29 mM picoxystrobin, 1.36 mM pyraclostrobin). Data are mean ( $\pm$  SE),  $n = 8$ .

**Table 2.** Parameters, significance and goodness of fit of a single rectangular hyperbola [ $y = a \times g_{sw} / (b + g_{sw})$ ] to the relationships of the rates of net photosynthesis,  $A_n$ , and transpiration,  $E$ , and the intercellular carbon dioxide concentration,  $c_i$ , with the rate of stomatal conductance to water,  $g_{sw}$ , of the mid-portion of the second fully expanded leaf on the main stem of well-watered or severely water-stressed wheat plants. Measurements were taken at two soil water potentials ( $-0.1$  and  $-1.5$  MPa) 1, 3 and 7 days after application of distilled water, distilled water + acetone (50 + 50 by volume) or four different strobilurin fungicides at half field rate ( $0.625 \text{ g dm}^{-3}$ ).  $n = 144$

Variable	$a$	$b$	$r$	$P$
$A_n$	$35 \pm 1$	$199 \pm 7$	0.987	<0.001
$E$	$11 \pm 1$	$489 \pm 26$	0.992	<0.001
$c_i$	$258 \pm 4$	$37 \pm 3$	0.873	<0.001

(Table 4). Consequently, the ratio  $F_v/F_m$  (an indicator of the maximum quantum efficiency of PSII) was also reduced in strobilurin-treated plants relative to plants treated with the blank solvent or epoxiconazole ( $P < 0.025$  in all comparisons).

#### 4 DISCUSSION

All of the strobilurin fungicides examined reduced the rate of stomatal conductance to water,  $g_{sw}$ , of leaves of wheat, barley and soya plants. Concomitantly, plants treated with strobilurin fungicides had a lower rate of transpiration,  $E$ , a lower intercellular carbon dioxide concentration,  $c_i$ , and a lower

net rate of photosynthesis,  $A_n$ , compared with control plants or plants treated with a triazole fungicide (epoxiconazole). Effects on the photosynthetic parameters of wheat leaves of two of the strobilurin fungicides increased with increasing rates of application, up to 0.5 mM. Above 0.5 mM, no further reductions in photosynthetic parameters were observed. The magnitude and duration of the reduction in photosynthetic parameters was different for each of the five strobilurin fungicides tested. In addition, one of the strobilurin fungicides reduced dark respiration of wheat leaves, which is perhaps unsurprising given that strobilurin fungicides are known inhibitors of mitochondrial respiration in plants,<sup>4-6</sup> although mitochondria isolated from fungi are more susceptible.<sup>5</sup>

Perhaps the most striking effect of the strobilurin fungicides was the reduction in  $g_{sw}$  of treated leaves. The detailed criteria on which the movements of stomata are based are not well understood.<sup>22</sup> For this reason it is difficult to identify the mechanism by which strobilurin fungicides influence stomatal

**Table 3.** Water use efficiency of the second fully expanded leaf on the main stem of well-watered or severely water-stressed wheat plants evaluated 1, 3 and 7 days after application of distilled water (control), distilled water + acetone, 50 + 50 by volume (blank), or one of four strobilurin fungicides at half field strength ( $0.625 \text{ g dm}^{-3}$ ) in distilled water + acetone, 50 + 50 by volume. Data are mean  $\pm$  SE,  $n = 4$

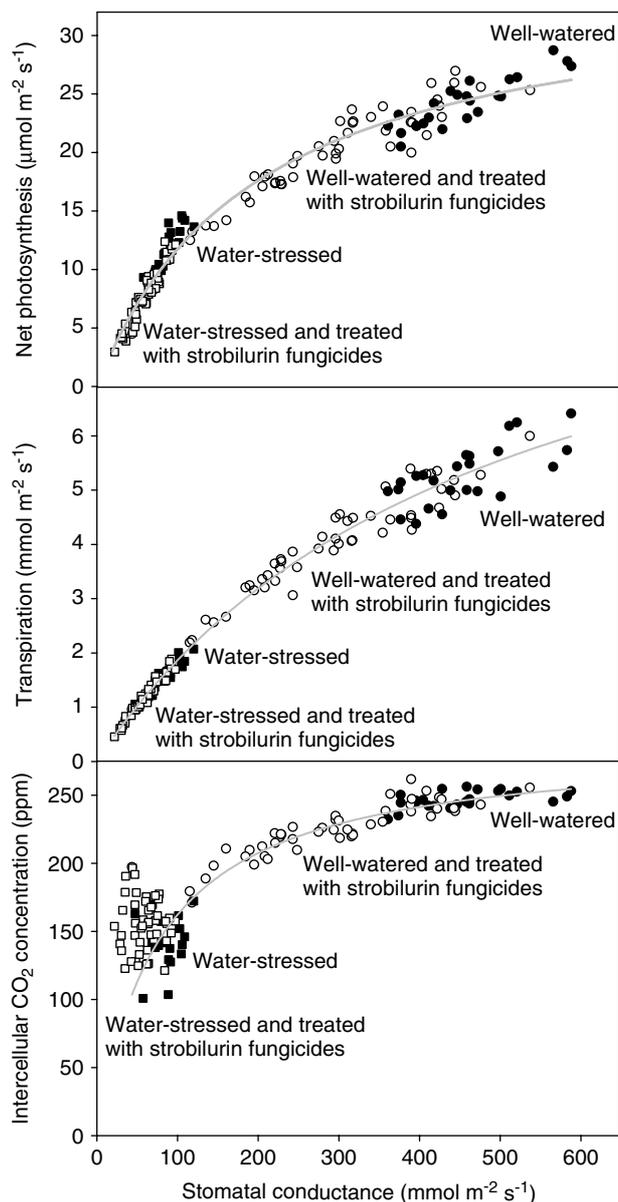
Treatment	1 DAS	3 DAS	7 DAS
	WUE [ $\text{mol H}_2\text{O} (\text{mol CO}_2)^{-1}$ ] <sup>a</sup>		
<i>Well watered</i>			
Control	208 $\pm$ 6a	227 $\pm$ 7a	215 $\pm$ 5a
Blank	220 $\pm$ 9a	218 $\pm$ 8a	219 $\pm$ 9a
Strobilurin 1	198 $\pm$ 10a	210 $\pm$ 20a	209 $\pm$ 10a
Strobilurin 2	205 $\pm$ 7a	181 $\pm$ 11b	199 $\pm$ 6b
Strobilurin 3	186 $\pm$ 5b	205 $\pm$ 9a	225 $\pm$ 10a
Strobilurin 5	182 $\pm$ 9b	196 $\pm$ 10b	205 $\pm$ 4a
<i>Severe water stress (-1.5 MPa)</i>			
Control	129 $\pm$ 9c	142 $\pm$ 7c	128 $\pm$ 8c
Blank	154 $\pm$ 7d	140 $\pm$ 8c	143 $\pm$ 7c
Strobilurin 1	149 $\pm$ 6c	163 $\pm$ 5c	143 $\pm$ 6c
Strobilurin 2	145 $\pm$ 5c	191 $\pm$ 5b	147 $\pm$ 6c
Strobilurin 3	168 $\pm$ 9d	154 $\pm$ 14c	148 $\pm$ 7c
Strobilurin 5	160 $\pm$ 3d	146 $\pm$ 4c	159 $\pm$ 15d

<sup>a</sup> Values in the same column followed by a different letter are significantly different at  $P = 0.05$ .

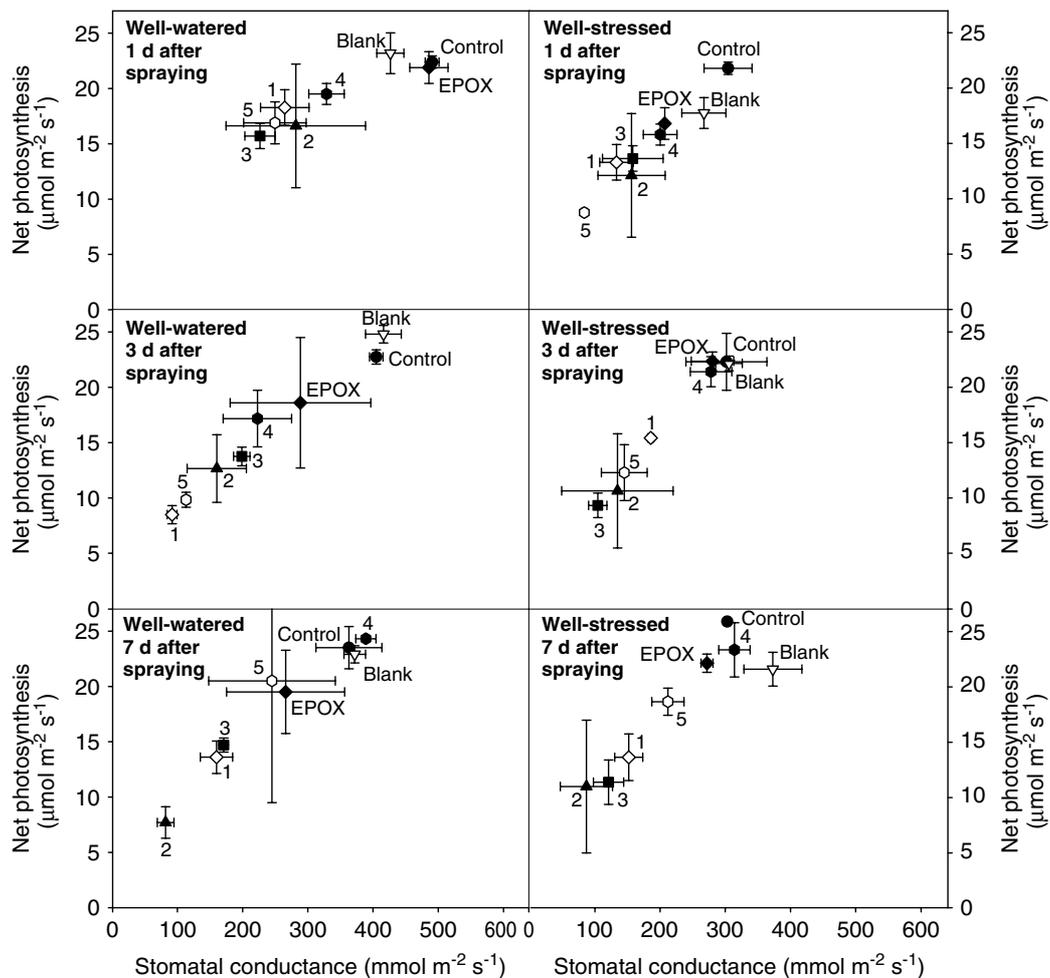
**Table 4.** Minimal,  $F_0$ , maximal,  $F_m$ , and variable chlorophyll fluorescence,  $F_v$ , and  $F_v/F_m$  (maximum quantum efficiency of PSII photochemistry) for the centre portion of the centre leaf on the first trifoliolate of soya plants measured 2 days after application of distilled water + acetone, 50 + 50 by volume (blank), epoxiconazole or two different strobilurin fungicides, picoxystrobin and pyraclostrobin, at full field strength ( $1.25 \text{ g dm}^{-3}$ ) in distilled water + acetone, 50 + 50 by volume. Data are mean  $\pm$  SE,  $n = 4^a$

Treatment	$F_0$	$F_m$	$F_v$	$F_v/F_m$
Blank	262 $\pm$ 30a	1182 $\pm$ 24a	262 $\pm$ 15a	0.78 $\pm$ 0.02a
Epoxiconazole	298 $\pm$ 40a	1153 $\pm$ 66a	298 $\pm$ 20a	0.74 $\pm$ 0.03a
Strobilurin 1	324 $\pm$ 48b	1074 $\pm$ 44b	324 $\pm$ 24b	0.69 $\pm$ 0.03b
Strobilurin 2	317 $\pm$ 30b	1050 $\pm$ 55b	317 $\pm$ 15b	0.69 $\pm$ 0.02b

<sup>a</sup> Values in the same column followed by a different letter are significantly different at  $P = 0.05$ .



**Figure 4.** Relationships between the rate of stomatal conductance to water and the rate of net photosynthesis (top), the rate of net transpiration (middle) and the intercellular carbon dioxide concentration (bottom) of the mid-portion of the second fully expanded leaf on the main stem of well-watered or severely water-stressed wheat plants. Measurements were taken at two soil water potentials ( $-0.1$  and  $-1.5 \text{ MPa}$ ) 1, 3 and 7 days after application of distilled water, distilled water + acetone (50 + 50 by volume) or four different strobilurin fungicides at half field rate ( $0.625 \text{ g dm}^{-3}$ ). Fitted curves are single rectangular hyperbolae where  $y = a \times g_{sw} / (b + g_{sw})$ ,  $n = 144$ .



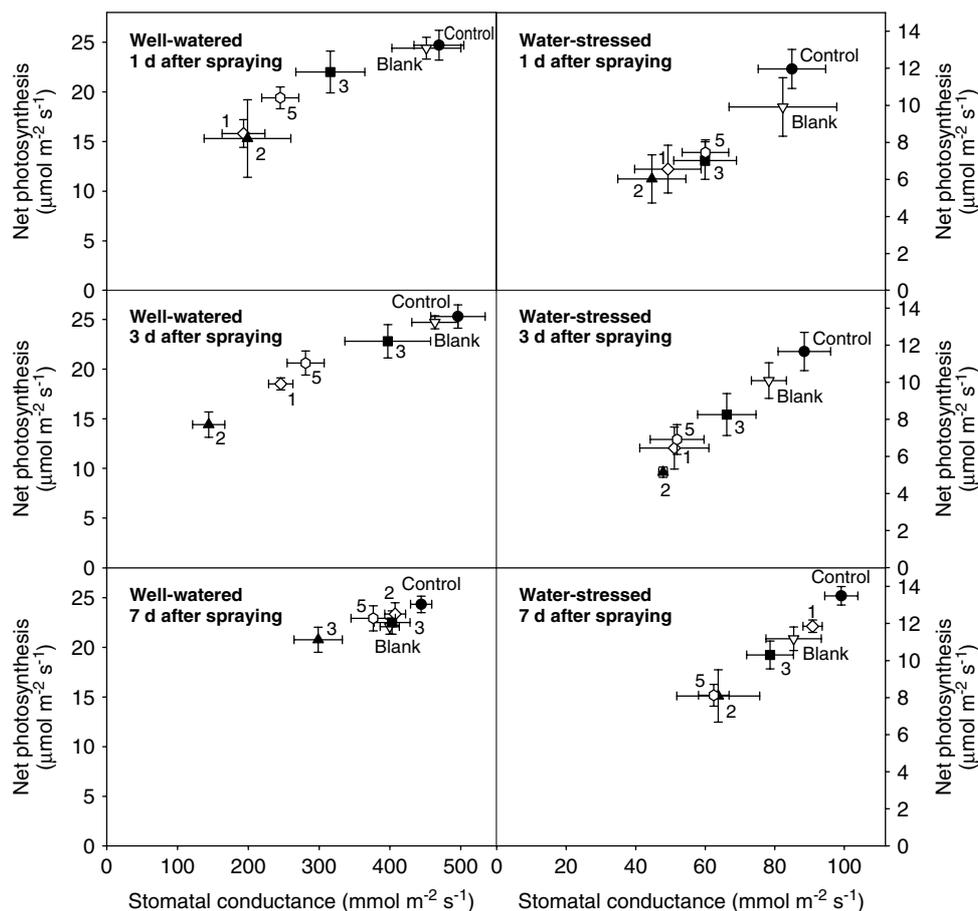
**Figure 5.** Rate of net photosynthesis against rate of stomatal conductance to water of the second fully expanded leaf of the main stem of wheat plants from well-watered or lightly water-stressed plants. Plants were evaluated 1, 3 and 7 days after application of distilled water (control), distilled water + acetone, 50 + 50 by volume (blank), epoxiconazole (EPOX) or one of five strobilurin fungicides (1, picoxystrobin; 2, pyraclostrobin; 3, azoxystrobin; 4, kresoxim-methyl; 5, trifloxystrobin) at half field strength ( $0.625 \text{ g dm}^{-3}$ ) in distilled water + acetone, 50 + 50 by volume. Data are mean ( $\pm$  SE),  $n = 3$ .

conductance, a process which is tightly coupled with photosynthesis.<sup>23</sup> It is possible that stomata are responding to strobilurin-induced changes in mesophyll photosynthesis<sup>24</sup> either by sensing changes in  $c_i$ <sup>12</sup> or by responding to the pool size of an as yet unidentified C-fixing substrate.<sup>23</sup> It is also possible that the effects of strobilurin fungicides are mediated via ABA-based chemical signalling.<sup>25</sup> The present authors propose a simple hypothesis to explain the strobilurin-induced reduction in  $g_{sw}$ : strobilurin fungicides act directly on stomata by binding to the Qo site of the cytochrome  $bc_1$  complex in guard cell mitochondria.<sup>1-3</sup> It has been proposed that the osmotic gradient across guard cell membranes is limited by guard cell ATP production.<sup>26,27</sup> Reduced production of ATP by strobilurin-inhibited guard cell mitochondria may impair functioning of the usual metabolism within guard cells and cause them to reduce turgor. The consequent reduction in stomatal aperture lowers  $c_i$  and  $A_n$  by restricting diffusive transport of carbon dioxide. This hypothesis may explain the reduction in  $g_{sw}$  without the need to invoke complex chemical signalling and feedback pathways.

However, it is likely that the response of stomata to strobilurin fungicides is complex, and further research is required to elucidate the different biochemical pathways involved.

The strobilurin fungicides reduced the ratio  $F_v/F_m$  (an indicator of the quantum yield of photosystem II) of treated leaves of soya plants. This finding indicates that strobilurin fungicides inhibit photosynthesis directly and perhaps independently of the effects on stomata. A testable hypothesis that may explain this effect is that strobilurin fungicides block transport of electrons between PSII and PSI by binding to the Q<sub>i</sub> site of the chloroplast cytochrome  $bf$  complex. However, as has previously been noted,<sup>3</sup> it is possible that other quinone-mediated reactions are inhibited by strobilurin fungicides. It is also possible that the effects on chlorophyll fluorescence are secondary and arise from inhibition of carboxylation reactions or respiration, since there is a close association between photosynthesis and mitochondrial metabolism.<sup>28-30</sup>

Strobilurin fungicides confer long-lasting disease control<sup>1</sup> and only degrade slowly;<sup>31</sup> however, the photosynthetic parameters of plants treated with

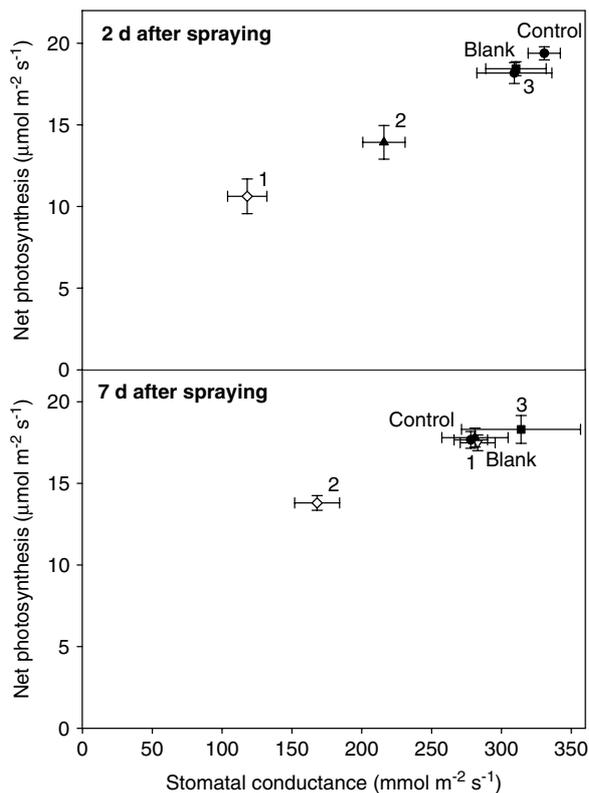


**Figure 6.** Rate of net photosynthesis against rate of stomatal conductance to water of the second fully expanded leaf on the main stem of wheat from well-watered or severely water-stressed plants. Plants were evaluated 1, 3 and 7 days after application of distilled water (control), distilled water + acetone, 50 + 50 by volume (blank) or one of four strobilurin fungicides, (1, picoxystrobin; 2, pyraclostrobin; 3, azoxystrobin; 5, trifloxystrobin) at half field strength (0.625 g dm<sup>-3</sup>) in distilled water + acetone, 50 + 50 by volume. Note different scales on both axes for water stressed. Data are mean (± SE), n = 4.

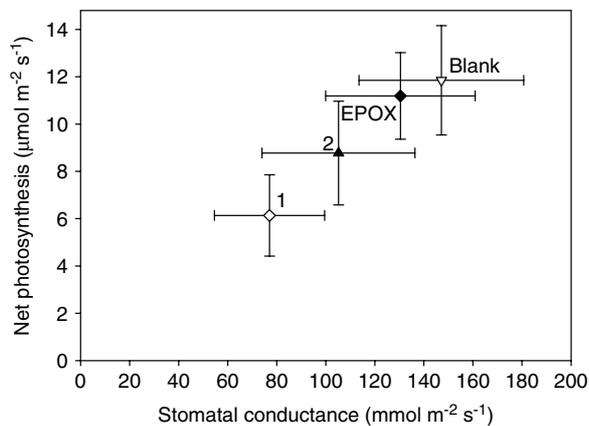
some of the strobilurin fungicides recovered to near the level of control plants within 7 days. It may be that recovery by the plants is partially due to upregulation of the alternative oxidase (AOX) pathway, as occurs in fungi in response to strobilurins.<sup>32,33</sup> Upregulation of the AOX pathway has been shown to enhance photosynthetic electron transport in leaves of wheat grown under drought conditions.<sup>34</sup> However, a possible purpose of the AOX pathway is to act as a sink for reducing power under conditions of environmental stress, resulting in a proportion of the reducing power being dissipated as heat instead of being used to reduce NADH. Upregulation of the AOX pathway alone is therefore unlikely to be responsible for the substantial recovery of the rates of  $g_{sw}$  and  $A_n$  of strobilurin-treated plants. Differences in the activity and persistence of strobilurin fungicides are governed mainly by their biokinetic properties, including lipophilicity, potential for translaminar activity and xylem systemicity.<sup>1,17</sup> Additional factors, such as photodegradation of strobilurins<sup>31</sup> and the reversible nature of strobilurin binding,<sup>1</sup> may contribute to the recovery of the photosynthetic parameters of strobilurin-treated plants.

There is limited scope for using strobilurins to improve the water status of crops grown under conditions of water deficit, as, although the WUE (the ratio of  $E$  to  $A_n$ ) of well-watered plants was improved slightly by application of strobilurin fungicides, the WUE was reduced in water-stressed plants. This may be related to the non-linear relationship between  $E$  and  $A_n$ , which dictates that, when  $E$  is already low owing to stomatal closure in response to water stress, reducing  $E$  further by applying strobilurin fungicides has a negative impact on  $A_n$ .

Non-linear relationships between photosynthesis and stomatal conductance have been found by several other workers.<sup>14,35,36</sup> Progressively greater inhibition of photosynthesis with decreasing leaf water content may be a result of decreased relative importance of  $g_{sw}$  and increased importance of metabolic limitation, for example limitation of ribulose bisphosphate (RuBP) regeneration.<sup>36</sup> In this case, applying strobilurin fungicides to water-stressed plants would not be expected to improve WUE, especially if strobilurin fungicides further reduce the production of ATP by inhibiting mitochondrial respiration.



**Figure 7.** Rate of net photosynthesis against rate of stomatal conductance to water of the third fully expanded leaf on the main stem of barley plants. Plants were evaluated 2 and 7 days after application of distilled water (control), distilled water + acetone, 50 + 50 by volume (blank), picoxystrobin (1), pyraclostrobin (2) or azoxystrobin (3) at half field strength ( $0.625 \text{ g dm}^{-3}$ ) in distilled water + acetone, 50 + 50 by volume. Data are mean ( $\pm$  SE),  $n = 4$ .



**Figure 8.** Rate of net photosynthesis against rate of stomatal conductance to water of the centre portion of the centre leaflet on the first trifoliate leaf of soya plants measured 2 days after application of distilled water + acetone, 50 + 50 by volume (blank), epoxiconazole (EPOX) or two different strobilurin fungicides (1, picoxystrobin; 2, pyraclostrobin) at field strength ( $1.25 \text{ g dm}^{-3}$ ) in distilled water + acetone, 50 + 50 by volume. Data are mean ( $\pm$  SE),  $n = 4$ .

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