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# Anti-oxidative and anti-senescence effects of the strobilurin pyraclostrobin in plants: A new strategy to cope with environmental stress in cereals

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## ABSTRACT

In addition to its broad spectrum fungicidal activity, the strobilurin pyraclostrobin had positive effects on the crop yield in the absence of pathogen challenge. This physiological effect on the plants was especially apparent under conditions of environmental stress. We have observed that pyraclostrobin prevented both symptom development and yield reduction by physiological leaf spot in barley. Foliar application of pyraclostrobin reduced the production of reactive oxygen intermediates in barley leaf tissues by more than 50% and activated the plant antioxidative system. In addition, pyraclostrobin treatment prevented the release of stress-induced ethylene and premature senescence. Since the physiological leaf spot disease and other environmental stresses are caused by changes in the genetic and metabolic regulation of reactive oxygen intermediates resulting in membrane-leakage, cell death or premature senescence, we postulate that the anti-oxidative and anti-senescence effects of pyraclostrobin are responsible for its ability to improve stress tolerance in plants.

## INTRODUCTION

Mitigating plant stress, whether from fungal pathogens or environment factors, is critical to maximising crop yield performance. Today, fungal pathogens can be effectively controlled by broad-spectrum fungicides such as the strobilurin class. Apart from their fungicidal effects, strobilurins such as kresoxim-methyl and pyraclostrobin (*F500®*) can cause long-term changes in the metabolism and growth of the treated plants resulting in higher biomass and yield (Köhle *et al.*, 1997; Glaab & Kaiser, 1999). Moreover, increases in biomass paralleled proportional increases in starch and protein. We believe that strobilurin treatment widens the critical bottlenecks for carbon as well as nitrogen assimilation. A partial inhibition of the respiration in leaf tissue caused by kresoxim-methyl decreased the $CO_2$-compensation point (Köhle *et al.*, 1997) and stimulated nitrate reductase activity (Glaab & Kaiser, 1999). Additionally, strobilurin treatment has been observed to alter the level of several phytohormones and delay plant senescence (Grossmann & Retzlaff, 1997).

A leaf spot disorder of barley with unknown aetiology has become an important issue in various regions of Europe (e.g. up to 40% or 3 t/ha yield loss in Southern Germany). This disorder is referred by several names such as physiological leaf spots (PLS), nonparasitic necrosis, genetic necrosis, and tar spots. PLS symptoms are characterised by necrotic spotting in the uppermost four leaves and are not caused by a microbial pathogen, but by unknown genetic or abiotic stress factors. However, in some, but not all, regions, *Ramularia collo-cygni* is considered to contribute to the PLS complex (Sachs, 2002).

Less is known about the causal mechanism of nonparasitic necrosis in plants compared to necrosis following a hypersensitive resistance response caused by pathogens (Jabs & Slusarenko, 2000). Nevertheless, Jabs *et al.*, (1996) found some striking similarities between pathogen-dependent hypersensitive response and necrotic lesions induced by excessive light or artificial oxidative stress on *Arabidopsis* plants. Both types of lesions express the same genetic and histochemical markers on the macroscopical and microscopical level, such as callose deposition, production of reactive oxygen intermediates and the increase of stress-related enzymes. Thus, plants respond to abiotic and biotic stress with similar physiological mechanisms. Reactive oxygen intermediates (ROI) seem to play an important role inducing programmed cell death, membrane-leakage, ethylene release, and local necrosis or premature senescence (Jabs & Slusarenko, 2000; Overmyer *et al.*, 2000). Recently, evidence has also been presented for the involvement of oxidative stress in the formation of physiological leaf spot. ROI accumulation in barley leaves was correlated with the severity of PLS as well as with the cultivar-specific susceptibility to PLS in the field (Wu & von Tiedemann, 2002a). The purpose of this study was achieve a better understanding of the altered plant stress responses after pyraclostrobin treatment under field conditions.

## MATERIALS AND METHODS

Table 1. Fungicides used for applications

| Code | Active ingredient (g/litre) |
|---|---|
| PYR+EPX | pyraclostrobin (133) + epoxiconazole (50) |
| PYR | pyraclostrobin (250) |
| EPX | epoxiconazole (125) |
| Strob. 1 | Strobilurin 1 (50% w/w) |
| Strob. 2 | Strobilurin 2 (250) |
| Strob. 3 | Strobilurin 3 (250) |

For the Physiological leaf spot field trial in Frankendorf, Bavaria, in 2000, the highly sensitive winter barley *cv.* Anthere and the less sensitive *cv.* Gunda were used. Fungicide treatments were applied at GS 39 or GS 51 as follows: PYR+EPX (1.75 litres/ha). All plots were pre-treated with 1.5 litres/ha of Fortress Top (quinoxyfen 67 g/litre; fenpropimorph 250 g/litre) at GS 31. Letters above the columns indicate significant differences between treatments ($P<0.05$, LSD).

For determination of ozone injury, spring barley (*cv.* Scarlett) was grown under glasshouse conditions. Fungicide treatments were applied as foliar sprays (0.1% formulated products) at GS 30 3 days prior to a 2-day ozone fumigation period (150-180 ppb, 7h/d). Ozone injury was determined as described (Wu & von Tiedemann, 2002b; n = 10, $P<0.05$, LSD).

For determination of superoxide production and SOD activity, spring barley (*cv.* Scarlett) was grown under glasshouse conditions. Fungicide treatments were applied as consecutive foliar sprays (0.1% formulated products) at GS 32 and 39. Examinations were performed at GS 55 and 69 as described (Wu & von Tiedemann, 2002a; P<0.05).

For ethylene determination, young wheat plants (*cv.* Kanzler) were raised in vermiculite substrate under controlled environmental conditions. Fungicide treatments were applied as foliar sprays at GS 12-13 at 3days or 2 hours before stress treatment as follows (litres/ha): PYR+EPX (1.5); PYR (0.8); Strob. 1 (0.25 kg/ha); Strob. 2 (0.8); Strob. 3 (0.8). The stress regimen consisted of incubating detached shoots at 30°C and 60% humidity for 30 min. Ethylene release was determined as described (Grossmann & Retzlaff, 1997).

## RESULTS

### Inhibition of physiological leaf spot in barley by pyraclostrobin treatment

Under field conditions, treatment with the fungicide mixture OPERA® (PYR+EPX), containing pyraclostrobin and epoxiconazole, prevented development of PLS symptoms (Fig. 1A). A formulation without a.i. did not reduce leaf spot symptoms (data not shown). The development of PLS necrotic lesions was accompanied by a premature loss of chlorophyll and reduction of photosynthesis in the uppermost leaves (data not shown), resulting in a yield reduction in untreated plots of up to 2.2 t/ha (Fig. 1B). The barley cultivar Anthere, which is highly susceptible to PLS, responded more significantly to the fungicide treatment, strengthening the hypothesis that pyraclostrobin treatment ameliorates oxidative stress leading to nonparasitic leaf spots.

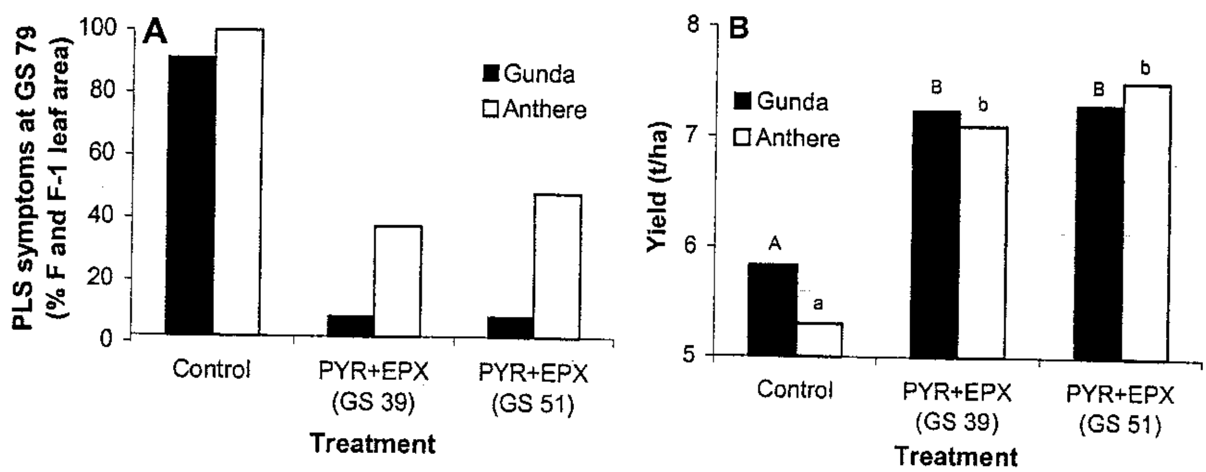


Figure 1. Effects of fungicides on Physiological Leaf spot symptoms in F and F-1 leaves (A) and on yield (B).

### Anti-oxidative effects by pyraclostrobin treatment

Fungicide treatment protected barley plants against artificial oxidative stress, such as ozone injury (Fig. 2; $p < 0.05$). Pyraclostrobin was most effective and reduced ozone damage by more than 90%.

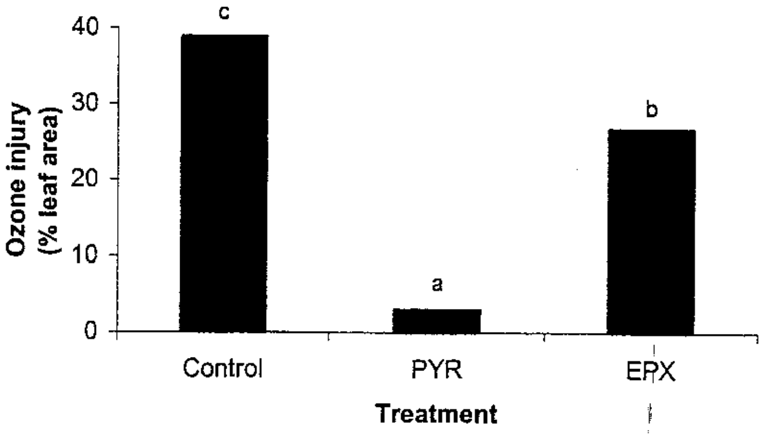


Figure 2. Effects of fungicides on ozone tolerance of barley leaves.

Furthermore, fungicide treatments to barley plants at GS 32 and 39 induced a sustained reduction of superoxide production even when evaluated at the advanced GS 55 ($P<0.01$, Fig. 3A). Superoxide production was reduced by about 50% by pyraclostrobin, while epoxiconazole was less efficient. The differences between fungicides in reducing ROI production became smaller at GS 69, but pyraclostrobin was still most effective (data not shown). In general, lowered ROI production in barley leaves was directly correlated with the protection of young barley plants from ozone damage by these fungicides.

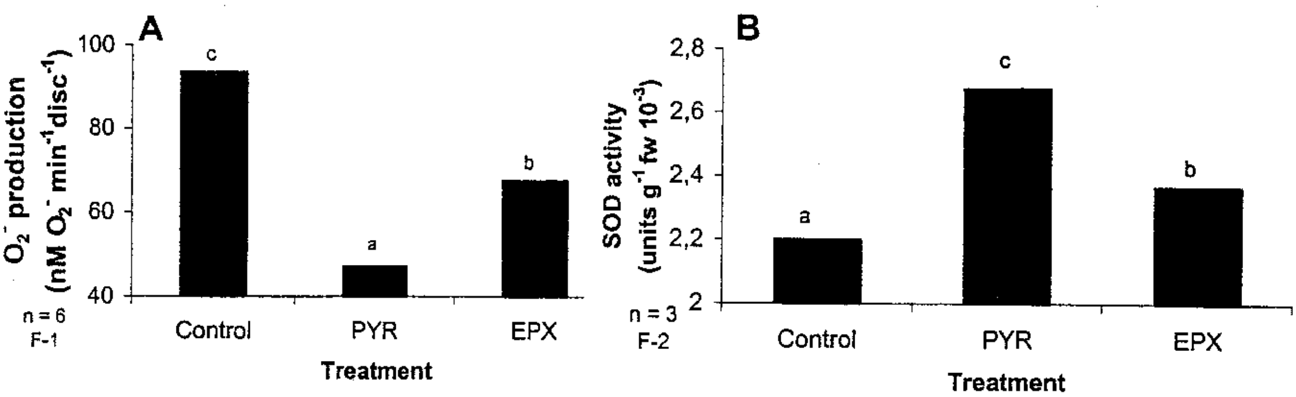


Figure 3. Effects of fungicides on superoxide production (A) and on SOD activity (B) of barley ( $P<0.05$).

Plants exhibiting increased tolerance to oxidative stress typically possess with increased activities of anti-oxidative enzymes, such as superoxide dismutases (SOD), catalases and peroxidases (Smirnoff, 1998). SOD activity in leaves is the primary scavenger for superoxide radicals and decreases in ageing leaf tissues, which is consistent with the increase of ROI production during plant senescence. Treatment with pyraclostrobin increased superoxide-scavenging SOD activity in barley leaves at GS 55 and was superior to the effects of epoxiconazole (Fig. 3B), which is in agreement with the strong reduction of superoxide production by pyraclostrobin. Even at more mature growth stages (GS 69) the enhancement of SOD activity by pyraclostrobin over the untreated control and other fungicides was observed (data not shown). Interestingly, this activation of the antioxidative system by pyraclostrobin treatment was found to precede the development of physiological leaf spot symptoms in a winter barley field trial (Köhle *et al.*, in press), indicating that oxidative stress is a cause rather than a consequence of this physiological disorder.

## Anti-senescence effects by pyraclostrobin treatment

In addition to ROI production, ethylene release is a rapid plant response to oxidative stress. Ethylene itself is required for sustained ROI production, which drives cell death propagation (Overmyer *et al.*, 2000). Pyraclostrobin treatment resulted in a strong reduction of ethylene production after a short-term drought stress regimen (Fig. 4). Pyraclostrobin applied only 2 hours before stress regimen resulted in more than 80% inhibition of ethylene release indicating a rapid uptake strong intrinsic activity of pyraclostrobin. Although inhibition of ethylene release seems to be a more general physiological side effect of strobilurins (Grossmann & Retzlaff, 1997), other strobilurin-containing fungicides were less effective and showed a delayed onset of the ethylene release inhibition. Even when applied 3 days before the stress regimen, pyraclostrobin treatment reduced ethylene formation for 80 h by more than 50% (Fig. 4B), indicating once more the excellent bioavailibility of pyraclostrobin.

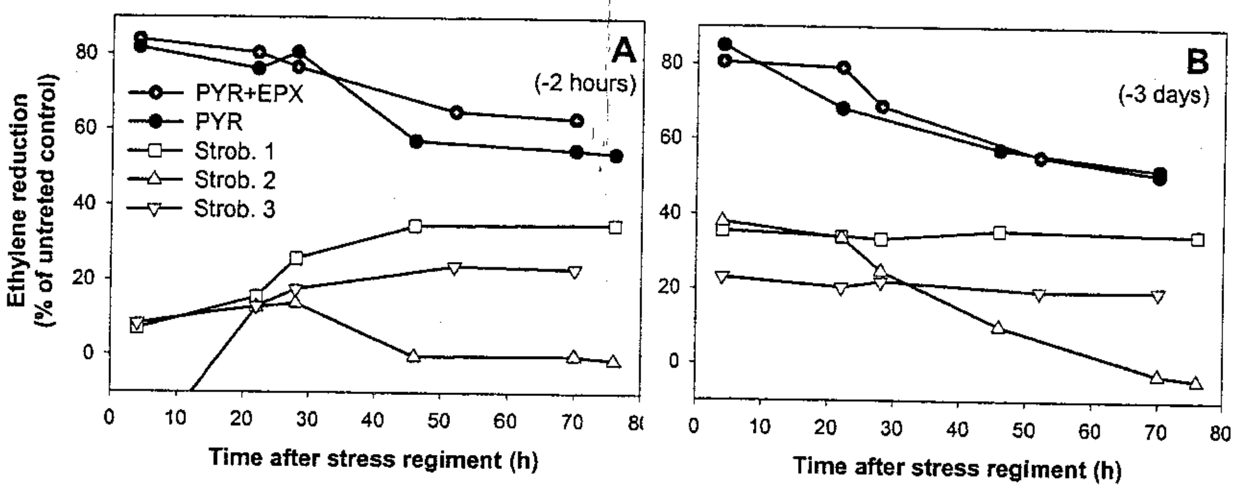


Figure 4. Effects of fungicides on ethylene release from detached and stressed wheat shoots. Fungicide treatments were applied as foliar spray to wheat plants (A) 2 hours or (B) 3 days before stress treatment.

## CONCLUSIONS

The physiological leaf spot disease and other environmental stresses are caused by changes in the genetic and metabolic regulation of ROIs (Wu & von Tiedemann, 2002a). ROIs form a feedback amplification cycle in concert with ethylene signaling, resulting in cell death, premature senescence and yield reduction (Overmyer *et al.*, 2000). Therefore, we postulate that both the strong anti-oxidative and anti-senescence effects of pyraclostrobin contribute to its excellent efficacy against PLS and to improved stress tolerance in cereals (Fig. 5). This helps plants to prolong the duration of corn filling and ensures optimal maturation and yield.

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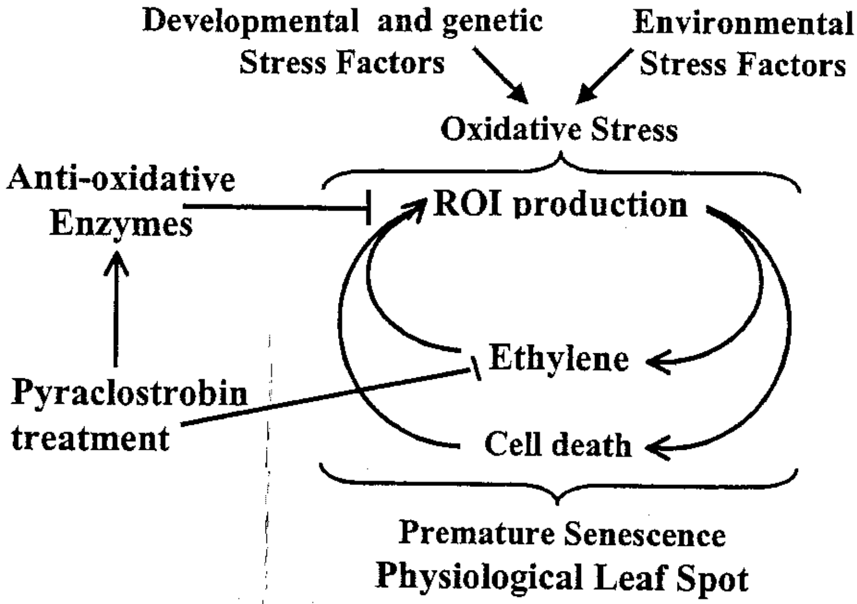


Figure 5. Hypothetical Model for the Action of pyraclostrobin treatment against oxidative plant stress.

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