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RESEARCH PAPERS

Efficacy of foliage fungicides against eyespot of winter wheat in Northern Italy

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Summary. The efficacy of foliage fungicide applications against eyespot of soft wheat cv. Serio was evaluated under natural *Oculimacula* infection in an experimental area in the Po Valley (Northern Italy). The fungicide treatments prochloraz, prochloraz + propiconazole, and trifloxystrobin + cyproconazole were applied in the years of 2006 through to 2009. Seeds were also treated with a formulated product based on guazatine. All foliage fungicides were applied at the stem extension growth stage (Zadoks growth stage 30–32), and at the manufacturer recommended rates. All tested treatments reduced the disease severity compared with untreated control. Prochloraz alone and particularly in combination with propiconazole gave the greatest efficacy in reducing eyespot. All treatments increased grain yield in 2006 and 2008. The effects of treatments on some yield parameters were also examined.

Key words: soft wheat, *Oculimacula*, prochloraz, propiconazole, chemical control.

Introduction

The eyespot disease, caused mainly by *Oculimacula acuformis* (Boerema, Pieters & Hamers) Crous & Gams, and *O. yallundae* (Wallwork & Spooner) Crous & Gams (formerly known as *Tapesia acuformis* and *T. yallundae*), is one of the components of the foot and root disease complex of winter cereals in temperate areas. The other components are brown foot rot, caused by *Fusarium* species and *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem, sharp eyespot caused by *Rhizoctonia cerealis* van der Hoeven, and take-all caused by *Gaeumannomyces graminis* von Arx & Olivier var. *tritici* Walker. The main sources of *Oculimacula* primary inoculum are infested crop residues, where the pathogen survives as mycelium in the inter-crop period (Wiese, 1987). Survival of the fungus depends on several factors, such as nature and position of crop residues in the soil profile, presence or absence of antagonistic, competitive biota, and climatic conditions (Matusinsky *et al*., 2009). Conidia are responsible of infection, their production is maximum when temperatures are near 10°C, and the disease does not occur below 0 or above 20°C. Conidia produced on infected debris and dispersed in rain-splash drops, can infect the coleoptiles of young plants from autumn to spring, within 15 h at temperatures between 6 and 16°C. The infection progresses through leaf sheaths before producing the typical eyespot lesions in the basal stem portions of plants, which can cause breaking and lodging later in the season. Secondary infections originating from conidia produced on plant lesions can occur. Mild winters and moist springs are favourable to the disease (Wiese, 1987).

In Italy, brown foot rot is the major component of the foot and root disease complex of durum and soft wheat (Balmas *et al.*, 2000; Innocenti *et al*.,

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2000b; Covarelli and Nicholson, 2002; Montanari *et al.*, 2006), and this disease causes severe yield losses. However, in the last 10 years eyespot has been increasingly observed in central and northern Italy (Covarelli and Santori, 2000; Innocenti *et al.*, 2000a; Covarelli and Nicholson, 2002), with 25–30% grain yield reductions occurring in cases of severe disease (Alvisi, personal communication). The two species *O. yallundae* and *O. acuformis* were found in Italian fields both with high incidence levels (Covarelli and Nicholson, 2002). Both pathogens cause identical symptoms, so they cannot be distinguished by visual assessment. They are, however, different in some aspects, including sensitivity to certain fungicides. Leroux *et al.* (2006), in experiments carried out in France, showed that *O. acuformis* had natural resistance to sterol 14α-demethylation inhibitors, and that *O. yallundae* had quantitative acquired resistance to triazoles (e.g. bromuconazole, epoxiconazole, and flusilazole). Moreover, acquired resistance to prochloraz was recorded in both species. Bateman (2002) in United Kingdom, did not observe resistance to prochloraz, even if a gradual loss of prochloraz efficacy after several years of application was observed probably due to *O. yallundae* lower sensitivity to this fungicide. No data are available, to our knowledge, on an effective biological control of the disease under field conditions, so chemical control is the only currently available disease management method for this disease. Responses of fungicide treatments are variable, depending not only on composition of the pathogen populations, as cited above, but also on cultivar susceptibility and edaphic and climatic conditions. In spite of severe losses, very few studies on chemical control of eyespot have been carried out under field conditions in Italy, although a 1-year study was conducted by Covarelli and Santori (2000) in central Italy. Therefore the aim of the present study was to evaluate the efficacy of some foliar fungicides against eyespot in the field carried out in a typical cereal-growing area of northern Italy, where severe natural *Oculimacula* infections occurred.

Materials and methods

Experimental site, treatments and sampling

The study was carried out at a farm located in a wheat-growing area in the Po Valley, near Bologna (Northern Italy), on clay soil classified as Inceptisoil, Udertic Ustochrepts, Fine, Mixed, Mesic (FAO classification). Minimum tillage (chisel plough) was used, and the soft wheat cv. Serio, susceptible to Oculimacula disease, was sown in mid- October of each year (Table 2). The trial was set up in 2004, and in 2005 a severe attack of eyespot disease was observed. In March 2006 the first fungicide application against eyespot was performed (Table 2). Data reported in this paper refer to the period 2006 through to 2009. Wheat seeds were dressed with guazatine (Panoctine L, Makhteshim Agan, Bergamo, Italy) prior to sowing, and plants were treated with prochloraz (Sportak 45 EW, BASF, Cesano Maderno (MB), Italy), prochloraz + propiconazole (Bumper P, Makhteshim Agan), or trifloxystrobin + cyproconazole (Agora, Bayer CropScience) at product label rates (Table 1). Foliar fungicides were applied by pressurized knapsack sprayer (400 L ha^{-1}) once each growing season (Table 2) at the stem extension crop growth stage (Zadoks growth stage, ZGS, 30–32; Zadoks *et al.*, 1974), before secondary infections occurred. Weeds were controlled in spring by applying a mixture of the products Ariane II (fluroxipir 40 g L^{-1} + clopiralid 20 g L^{-1} + MCPA 200 g L^{-1}) at 4 L ha^{-1} with Topic 80 EC (clodinafop- propargyl 80 g L^{-1} + cloquintocet- mexyl 20 g L^{-1}) at 0.75 L ha^{-1}. Crop residues were left on or very close to the soil surface. Each plot (replicate) measured 2.5 × 10 m, and the experimental design was a randomized block with four replicates. Meteorological data (air temperature and precipitation) were provided by the Azienda Regionale Prevenzione Ambientale Emilia-Romagna (ARPA), Italy. To evaluate the effect of each treatment on eyespot, 25 plants were randomly collected at the late milk development growth stage (ZGS 79–80) along two diagonals per plot at each sampling date (Table 2), giving a total of 100 ear-bearing tillers per plot. Plots were harvested using a plot combine harvester, and grain yields (at 13% moisture content) were obtained (Table 2). Yield components, including thousand grain weight (TGW), specific weight and protein content, were measured using standard techniques.

Disease assessment

Tillers collected at growth stage ZGS 79–80 were assessed for disease after separating stems from sheaths. Eyespot was expressed as disease index (DI) for each plot: all tillers from the same plot were divided in four classes of severity (0, no symptoms; 1,

Table 1. Seed dressing and foliage fungicides used in trials performed during the period 2006–2009 in the Po Valley (Italy).

Active ingredient	Trade name and amount ($g\ L^{-1}$) of a.i. in product	Rate of product
Guazatine	Panoctine L (300)	2 mL kg^{-1}seed
Prochloraz	Sportak 45 EW (450)	1300 g ha^{-1}
Prochloraz + propiconazole	Bumper P (400 + 90)	1250 g ha^{-1}
Trifloxystrobin + cyproconazole	Agora (375 + 160)	400 g ha^{-1}

Table 2. Dates of sowing, application of fungicide treatments, sampling and harvesting in consecutive wheat crops.

Cropping year	Sowing	Treatment[a]	Sampling[b]	Harvesting
2005/06	10 Oct.	13 April	3 June	5 July
2006/07	7 Oct.	25 March	28 May	-[c]
2007/08	11 Oct.	4 April	6 June	7 July
2008/09	15 Oct.	27 March	29 May	9 July

[a] At ZGS 30-32.
[b] At ZGS 79-80.
[c] All plants lodged prematurely from a wind storm in 2007, so effects of treatments on disease and yields could not be assessed.

slight, lesions girdling less than half the stem circumference; 2, moderate, lesions girdling more than half the stem circumference, tissue still firm; 3, severe, lesions girdling more than half the stem circumference, tissue softened) (Scott and Hollins, 1974). These data were used to calculate the eyespot index (%) using the following formula: [(number of tillers with slight eyespot + 2 (number of tillers with moderate eyespot) + 3 (number of tillers with severe eyespot) × 100] / total number of tillers examined × 3. To confirm visual diagnosis, portions of stem tissues with eye lesions (1 cm long) were surface-sterilized for 1 min in 1% sodium hypochlorite, rinsed twice with sterile distilled water and dried on sterile filter paper. Tissue segments were then placed on 2% Bacto agar + 50 mg L^{-1} rifampicin in Petri dishes (Murray, 1992). Plates were incubated for 10 d at 15°C under near ultraviolet light (NUV), colonies were then sub-cultured on 0.2% Potato Dextrose Agar (PDA) + 50 mg L^{-1} rifampicin and incubated at 15°C. Isolated fungi were identified by conidial morphology in pure culture (Murray, 1992). No distinction was made between the two species of *Oculimacula*. Portions of tissues without eyespot symptoms were plated on PDA medium to verify the presence of other pathogens.

Statistical analyses

Percentage disease severity data are presented untransformed. Before analysis of variance, however, they were subjected to angular (arcsin) transformation (Snedecor and Cochran, 1980). Data were analyzed by Statistical Analysis System (SAS Institute Inc, Cory, NC, USA, 1997). They were subjected to two-way ANOVA, and when interactions were statistically significant ($P \leq 0.05$), Student Newman Keuls test (SNK) was performed to assess differences between means.

Results

Meteorological data

Air temperature and precipitation (Table 3) were favourable to eyespot over the whole period of observation. In particular, the wet spring periods in 2007 and 2009 were conductive to disease development.

Table 3. Total monthly precipitation (mm) and mean monthly temperature (°C) in the experimental area over the period of observation. Data provided by Azienda Regionale Prevenzione Ambientale (ARPA) Emilia- Romagna, Italy.

Month	2006		2007		2008		2009	
	mm	°C	mm	°C	mm	°C	mm	°C
January	5.6	0.4	22.2	4.9	42.4	4.5	60.8	2.0
February	5.4	3.9	40.8	6.4	16.2	4.5	52.6	4.2
March	20	7.5	105.4	9.2	23.4	8.7	87.4	8.6
April	21.4	12.3	23.4	14.9	45.0	12.6	83.2	13.5
May	41.6	17.1	45.4	19.2	47.0	17.7	72.2	19.3
June	7.4	21.7	73.6	22.5	121.6	21.6	49.2	21.5
October	13.4	15.7	103.6	13.5	37.8	15.3	59.2	13.3
November	43.4	8.9	16.8	6.9	151.4	9.0	74.2	9.6
December	19.1	4.7	46	2.6	55.3	3.7	62.5	2.1

Table 4. Mean eyespot indices (0–100) for wheat plants in plots treated with different fungicides during three growing seasons. Disease severity was assessed in plants collected at ZGS 79–80 each year. Percentage values were angular transformed before statistical analysis, but non-transformed means are presented here. Each datum is the mean of four replicates.

Foliage treatment	2006	2008	2009	Overall treatment means[a,b]
Untreated control	69.0	57.5	83.7	70.1 d
Prochloraz	37.8	20.6	60.4	39.6 b
Prochloraz + propiconazole	26.6	11.3	51.4	29.8 a
Trifloxystrobin + cyproconazole	37.9	39.4	75.7	51.0 c
Overall yearly means[b]	42.8 a	32.2 a	60.3 b	

[a] Foliage treatment and year factors were statistically significant ($P<0.01$), while the interaction between these was not ($P>0.05$).
[b] Means in the same column or line followed by different letters were significantly different according to the SNK test ($P<0.01$).

Eyespot severity, yield and thousand grain weight

Wheat plants affected by eyespot disease were found in plots of all of the treatments throughout the period of observation. The mean disease index (DI) values of untreated controls were 69% in 2006, 58% in 2008 and 84% in 2009 (Table 4). The presence of other foot, root and foliage diseases was very sporadic and always less than 3% incidence. In 2007 all plants lodged prematurely from a wind storm. Consequently, the microclimatic conditions were very favourable to the pathogen and the pressure of disease was very high, favoured also by the moist spring. It was not possible to assess the effect of treatments on the disease and it was not possible to harvest plants, so data from this year were not considered. To reduce primary inoculum on infected crop debris, in the late summer 2007 the soil was ploughed to a depth of 30 cm to bury the crop residues.

Concerning the effect of foliage fungicide treatments on eyespot DI, all of the fungicide treatments significantly reduced the eyespot DI, expressed as

Table 5. Mean grain yields (kg ha^{-1}) from wheat plots treated with different fungicides during three growing seasons.

Foliage treatment	2006[a,b]	2008[a,b]	2009[a,b]
Untreated control	597 a	610 a	540 a
Prochloraz	705 b	750 b	570 a
Prochloraz + propiconazole	677 b	865 c	540 a
Trifloxystrobin + cyproconazole	685 b	730 b	530 a

[a] Each datum is the mean of four replicates.
[b] Foliage treatment and year factors (P<0.01), and their interaction (P<0.05) were statistically significant. Means in the same column followed by different letters were significantly different according to the SNK test (P<0.05).

means over the 3 years, compared to the untreated controls. Prochloraz reduced DI by 44%, prochloraz + propiconazole by 58% and trifloxystrobin + cyproconazole by 27% (Table 4). Prochloraz + propiconazole was the most effective against the disease. Independently of treatments, the 2009 mean DI value (60%) was significantly greater than DI values of 2006 (43%) and 2008 (32%) (Table 4).

In 2006 and 2008, all the fungicide treatments increased grain yields compared with the untreated controls (Table 5). In 2006, the effect of treatments was similar, prochloraz increased yield by 18%, prochloraz + propiconazole by 13% and trifloxystrobin + cyproconazole by 14%. In 2008 prochloraz alone increased yield by 22%, and by 41% when combined with propiconazole. Trifloxystrobin + cyproconazole increased yield by 19%. In 2009 no significant differences were found between the fungicide treatments (Table 5).

The effect of the treatments on TGW, expressed as means of the 3 years, was similar and significantly greater than the untreated controls. Overall mean increases in TGW from the fungicide treatments were: prochloraz, 7.2%; prochloraz + propiconazole, 8.6%; and trifloxystrobin + cyproconazole, 9.2% (Table 6). Independent of treatment, the 2006 mean TGW (40.3 g) was significantly greater than those for 2008 (34.7 g) and 2009 (34.9 g). No statistically significant effects of the treatments were observed on specific weight or protein content over the whole observation period (data not shown).

Discussion

Eyespot was the only component of the foot and root disease complex of wheat plants, and foliage diseases was very sporadic, throughout the observation period. Eyespot was severe, with the mean disease index ranging from 58 to 84% in untreated plots. The cultural techniques adopted in this series of trials, including non-inversion of soil and continuous sowing of a susceptible wheat cultivar, were favourable to eyespot. This was previously demonstrated in a study carried out in a different cereal growing area in the Po Valley, where the effects of tillage and crop rotation on eyespot were examined (Montanari

Table 6. Mean thousand grain weights (g) from wheat plots treated with different fungicides during three growing seasons.

Foliage treatment	2006	2008	2009	Overall treatment means[a,b]
Untreated control	37.2	33.7	32.7	34.5 a
Prochloraz	41.0	35.4	34.6	37.0 b
Prochloraz + propiconazole	42.7	34.3	35.7	37.5 b
Trifloxystrobin + cyproconazole	40.4	35.7	36.9	37.7 b
Overall yearly means[b]	40.3 b	34.7 a	34.9 a	

[a] Each datum is the mean of four replicates.
[b] Foliage treatment and year factors were statistically significant (P<0.01), their interaction was not significant (P>0.05). Means in the same column or line followed by different letters were significantly different (P<0.05) according to the SNK test.

et al., 2006). In the present study, one-year plough adopted in autumn 2007, reduced eyespot disease in the following summer (2008), but this was not a durable control effect. In the 2009 assessment, eyespot was again very severe. Colbach and Meynard (1995) demonstrated that inoculum for a given year may not come from the previous year but from two years before.

Under the experimental conditions studied here, the three fungicide treatments prochloraz, prochloraz + propiconazole or trifloxystrobin + cyproconazole, controlled eyespot and increased grain yield in two out of three years of observation. Prochloraz alone or in combination with propiconazole was the most effective active ingredient against the disease. Prochloraz is one of the most commonly used fungicides against eyespot. However, its effectiveness is controversial. Bateman *et al.* (1995) and Bateman (2002) observed a gradual loss of prochloraz efficacy after several years of application in the United Kingdom. This was explained by selection in favour of strains with reduced sensitivity within the *O. acuformis* populations, although no resistance to this fungicide was found. Similarly, Ray *et al.* (2004) reported the inconsistency of prochloraz performance against eyespot in early sown winter wheat plots in United Kingdom, caused by its reliance on redistribution from foliage to stem bases by rainfall. Daniels and Lucas (1990) found that prochloraz was more effective when used as a protective than as a curative application, because of re-growth of the pathogen from structures within the host tissues not directly exposed to the fungicide. In accordance, Burnett *et al.* (1997) demonstrated that prochloraz applied at 30 ZGS, before secondary infections, was effective against *O. acuformis*. Babij *et al.* (2000) reported declining performance of prochloraz against eyespot used at two applications per year, over a total of 6 years. This decline in control was due to many factors including the rapid development of secondary eyespot infections encouraged by wet conditions and/or increasing frequency of less sensitive pathogen strains. Resistance to the fungicide was found in France by Leroux and Marchegay (1991) and Leroux *et al*. (2006).

In Italy very few studies on chemical control of eyespot have been carried out under field conditions. Covarelli and Santori (2000) in 1-year field experiment conducted in central Italy, showed that prochloraz was effective for reducing eyespot incidence. Our results, based on a 3-year study, confirm the efficacy of prochloraz against eyespot in a cereal growing area characterized by climatic conditions different from those of central Italy, which could potentially influence the efficacy of fungicides. Moreover, our data indicate that prochloraz was more effective against the disease when mixed with propiconazole. This mixture also gave the greatest increasing in grain yield in 2008.

In conclusion, this study has shown that prochloraz, prochloraz + propiconazole and trifloxystrobin + cyproconazole controlled eyespot in a typical cereal-growing area of northern Italy, and that prochloraz in combination with propiconazole, was the most effective treatment of those examined.

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