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Effect of foliar treatments to durum wheat on flag leaf senescence, grain yield, quality and DON contamination in North Italy

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3 **Effect of foliar treatments to durum wheat on flag leaf**
4 **senescence, grain yield, quality and deoxynivalenol**
5 **contamination in North Italy.**

6

7 Authors: Massimo Blandino*, Andrea Pilati, Amedeo Reyneri

8

9 Affiliation: Dipartimento di Agronomia, Selvicoltura e Gestione del Territorio,
10 University of Turin, via Leonardo Da Vinci 44, 10095 Grugliasco (TO), Italy.

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12 * Corresponding author: Tel: +39-011-6708895; fax +39-011-6708798.

13 E-mail address: massimo.blandino@unito.it

1 **Abstract**

2 Since the production of durum wheat in the drier areas of the Mediterranean Basin is
3 characterized by high variability in terms of yield and grain quality, there is also
4 considerable interest in developing durum wheat in the northern regions, where the
5 pedo-climatic conditions can offer the possibility of obtaining grain yields with higher
6 technological quality and stability. However, the climatic conditions in the northern
7 regions make durum wheat more prone to fungal foliar disease, particularly to
8 *Septoria Tritici Blotch* (*Septoria tritici* Rob.) and to *Fusarium Head Blight* (*Fusarium*
9 *graminearum* Petch and *F. culmorum* Sacc.), with the consequent occurrence of
10 DON in grains.

11 Field experiments have been conducted over two growing seasons at four sites in
12 North West Italy to evaluate the effect of fungicides and foliar nitrogen fertilizer
13 application on durum wheat yield and grain quality. Five combinations of foliar
14 application were compared at each site and each year (untreated control, azole
15 fungicide application at heading, strobilurin fungicide at the stem elongation stage
16 and/or at heading, the addition of a foliar N fertilizer to a fungicide programme). The
17 following parameters were analyzed: *Septoria Tritici Blotch* (STB) severity, flag leaf
18 greenness using a chlorophyll meter, grain yield, test weight, grain protein content,
19 ash content, vitreousness, *Fusarium head blight* (FHB) incidence and severity and
20 deoxynivalenol (DON) contamination. The collected data underline that the cultivation
21 of durum wheat at the climatic conditions of North Italy is actually risky and needs a
22 direct control of fungal disease, which would be able to reduce the development of
23 both foliar and head attacks. The double treatment, with a strobilurin application
24 during the stem elongation stage and azole at heading, results to be an essential

1 practice and showed advantages in terms of the delay of flag leaf senescence
2 (+27%), STB control (+31), FHB control (+11%), yield (+32%) and DON
3 contamination (-45%), compared to the untreated control. Other foliar treatments at
4 heading, such as strobilurin or foliar N fertilizer applications, do not seem to provide
5 any further advantage, for either grain yield or quality. No significant effect of
6 fungicide or foliar N fertilizer application was recorded on the protein or ash
7 concentration or vitreousness.

8 **Keywords:** durum wheat, fungicide, foliar nitrogen fertilizer, quality, deoxynivalenol.

1 **Introduction**

2 The Mediterranean Basin and North America are the main durum wheat (*Triticum*
3 *durum* Desf.) production areas in the world. Durum wheat is a minor cereal crop, but
4 of great relevance for areas like the Mediterranean, where it is commonly used for
5 human consumption: principally for pasta, but also for couscous, bulgur and bread. In
6 Italy, it is the main cereal crop, with more than 1.6 million ha producing 4 million tons
7 per year. Production is concentrated in southern and central Italy areas,
8 characterized by a warm winter and the frequent occurrence of drought, combined
9 with heat stress during grain filling (Corbellini *et al.*, 1997).

10 In recent years, improving durum wheat grain quality has become, one of the main
11 breeding and agronomic goals in many Mediterranean countries, due to the increase
12 in market demand for good quality grains (De Vita *et al.*, 2007). The protein content
13 and gluten quality have long been recognized as the most important factors that can
14 affect pasta-making properties (Kovacs *et al.*, 1997). Since production in South Italy
15 is characterized by high variability, in terms of yield and grain quality, mainly because
16 it is affected by the severity and irregularity of water stress and high temperatures
17 (Dunkeloh and Jacobeit, 2003), there is also considerable interest in durum wheat
18 cultivation in more temperate regions, such as northern Italy, where the climatic
19 conditions during grain filling could offer the possibility of obtaining grain yield with a
20 higher technological quality (Rharrabti *et al.* 2003a). In general, the cultivation of
21 durum wheat in cooler regions could favour heavy and more vitreous grains, with a
22 considerable increase in grain protein and gluten content (Gooding *et al.*, 2003), but
23 the grain quality could be negatively influenced by high percentages of ash
24 accumulated in the kernels (Rharrabti *et al.*, 2003a). On the other hand, cooler and

1 wetter weather conditions make durum wheat more prone to fungal disease (Pascale
2 *et al.*, 2002), particularly to Septoria Tritici Blotch (STB) and Fusarium Head Blight
3 (FHB).

4 STB, caused mainly by *Septoria tritici* Rob., has been associated with yield losses,
5 due to the reduction of the photosynthetic life of the canopy, especially in the flag
6 leaf, during grain filling (Puppala *et al.*, 1998). Moreover, both STB and FHB reduce
7 the yield and grain quality by causing shrivelled kernels and reduced test weight
8 (McKendry *et al.*, 1995; Parry *et al.*, 1995). *Fusarium graminearum* and *F. culmorum*,
9 the most important agents of FHB, are also the main causes of the accumulation, in
10 wheat kernels, of deoxynivalenol (DON), a mycotoxin of the trichotecenes group,
11 which is associated with serious mycotoxicosis in humans and animals (Bottalico and
12 Perrone, 2002). Pascale *et al.* (2002) observed that durum wheat varieties are
13 generally more prone to DON contamination than common wheat, particularly when
14 cultivated in more temperate regions. Since no durable, fully resistant wheat cultivars
15 exist at present (Snijders, 2004), the main strategy to protect durum wheat from
16 these foliar and head diseases is through fungicide applications, both during the stem
17 elongation stage and at anthesis (Ruske *et al.*, 2003b). Among the various fungicides
18 that are available, azole applications at anthesis have resulted to have a significant
19 effect on the reduction of the decline of green leaf area in flag leaves (Kettlewell *et*
20 *al.*, 1982) and on the increase of grain yield (Matthies and Buchenauer, 2000).

21 Fungicides containing triazole (the most important are bromuconazole,
22 epoxiconazole, metconazole, propioconazole, tebuconazole and tetraconazole),
23 imidazole (prochloraz) or triazolinthione (prothioconazole) active ingredients which
24 inhibit the biosynthesis of ergosterol, have proved to be the most active molecules

1 against FHB infection and DON contamination (Menniti et al., 2003; Paul et al.,
2 2008).

3 Strobilurin-fungicides were introduced as broad-spectrum fungicides in many
4 countries in the late 1990s. The inclusion of these fungicides in disease control
5 programmes for common wheat has been associated with extended flag leaf life and
6 increased grain yields (Dimmock and Gooding, 2002a) and grain protein content
7 (Dimmock and Gooding, 2002b). Strobilurins play an important role on the control of
8 several wheat leaf diseases such as STB, powdery mildew (*Erysiphe graminis*) and
9 rusts (*Puccinia* spp.), as reported by Oerke *et al.* (2001). Bayles (1999) reported that
10 strobilurins are able to prolong the duration of the green flag leaf area much longer
11 than previously available fungicides, such as azoles. Strobilurins have instead shown
12 poor efficacy against FHB caused by toxigenic *Fusarium* spp. (Pirgozliev *et al.*, 2002)
13 and often both *in vitro* and field studies have revealed an increase in DON
14 concentration following an application of these fungicides, compared to unsprayed
15 controls (Menniti *et al.*, 2003). To assure lower mycotoxin contamination in wheat
16 grain, the application of strobilurin fungicides is only recommended in a mixture with
17 azoles (Pirgozliev *et al.*, 2003).

18 Recently, the practice of adding a foliar nitrogen fertilizer to a fungicide programme at
19 heading has become widely diffused since it could increase the green flag leaf area
20 duration, maintain canopy longevity during grain filling and increase grain yield and
21 quality (Gooding *et al.*, 2007). Nitrogen applied at anthesis increased the protein
22 content in several experiments conducted with common wheat (Gooding and Davies,
23 1992; Bly and Woodard, 2003), because it is rapidly taken up and partitioned to the
24 grain. Moreover, the effect of foliar N fertilizer could be more consistent in higher

1 rainfall areas, for the higher nitrate leaching and potential yield (Gooding and Davies,
2 1992).

3 Since only a few data are at present available on durum wheat, the objective of this
4 study was to verify, in non inoculated conditions, the effect of foliar treatment
5 programmes, that include azole and strobilurin fungicide and N fertilizers, on durum
6 wheat disease control, yield, grain quality and safety, in order to implement growing
7 and defence tactics for this crop in northern Italy.

1 **Materials and Methods**

2 **Experimental site and treatments**

3 The experiment was carried out at four sites in North West Italy:

- 4 • S1: Cigliano (45° 18' N, 8° 01' E; altitude of 237 m.), in a shallow and sandy
5 soil, Typic Hapludalfs (USDA classification);
- 6 • S2: Marene (44° 40' N, 7° 44' E; altitude of 310 m.), in a deep and fertile
7 sandy soil, Typic Eutrochrepts;
- 8 • S3: Quargnento (44° 57' N, 8° 29' E; altitude of 121 m.), in a deep and acid
9 loamy soil, Aquic Frugiudalf;
- 10 • S4: Riva presso Chieri (44° 54' N, 7° 24' E; altitude 262 m.), in a sandy-
11 medium textured soil, Typic Udifluvents (USDA classification).

12 In the 2006-2007 period, the experiment was conducted at S2 and S3, while in the
13 2007-2008 period the experiment was conducted in all the sites mentioned
14 previously.

15 The compared treatments in each year and site were a combination of an azole
16 fungicide, a strobilurin fungicide and a liquid N fertilizer for foliar application. The
17 complete treatment schedule is summarized in table 1.

18 The following active ingredients or products were used:

- 19 ▪ Azole fungicide: mixture of prochloraz and cyproconazole (Tiptor[®] S, Syngenta
20 Crop Protection, Italy, formulation: emulsifiable concentrate (EC), applied at
21 0.36 and 0.048 kg active ingredient (a.i.) ha⁻¹, respectively;
- 22 ▪ Strobilurin fungicide: azoxystrobin (Amistar[®], Syngenta Crop Protection, Italy,
23 formulation: suspension concentrate), applied 0.25 kg active ingredient (a.i.)
24 ha⁻¹;

- 1 ▪ Liquid N fertilizers for foliar applications: YaraVita™ Last® N (Yara Italia S.p.A.,
2 composition: 312 g N l⁻¹, 25%), applied at 4.68 kg N ha⁻¹.

3 The treatments were assigned to experimental units using a completely randomised
4 block design with four replicates. The plot size was 8 x 1.5 m. The plots were seeded
5 after an autumn ploughing (30 cm) and disk harrowing to prepare a proper seedbed.
6 Planting was conducted in 12 cm wide rows at a seeding rate of 450 seeds m⁻². A
7 total of 170 kg N ha⁻¹ was applied to plots as granular ammonium nitrate fertilizer,
8 split in 50, 80 and 40 kg N ha⁻¹ between GS 31 and 39 and 55, respectively. The
9 variety used each year was Dakter [Eurodur and Semences de France (France) were
10 in charge of seed conservation]; which has been classified as high quality durum
11 wheat by the Arvalis Institut du Végétals. The fungicides or the leaf nitrogen fertilizers
12 were applied at 250 l ha⁻¹ with a 4 nozzle precision sprayer (T-Jet 110/04) using a
13 fine mist at a slow walk to ensure an effective coverage. The delivery pressure at the
14 nozzle was 324 KPa. The other main trial information for each year and site is
15 reported in table 2.

16 Grain yields were obtained by harvesting with a Walter Wintersteiger cereal plot
17 combine-harvester. A subsample was taken from each plot to determine the grain
18 moisture and test weight. The grain yield results were adjusted to a 120 g kg⁻¹
19 moisture content.

20 The harvested grains were accurately mixed, and 2 kg grain samples were taken
21 from each plot to analyse the grain protein content (GPC), ash content, vitreousness
22 and also to perform DON content analyses.

23

24

1 STB severity and flag leaf greenness

2 The severity of STB was assessed on 10 randomly chosen flag leaves per plot, after
3 a visual evaluation at the soft dough stage (GS 85), using a standard area diagram
4 (James, 1971).

5 A chlorophyll meter, Hydro N-Tester[®] (HNT) (Hydro-Agri, now Yara), was used to
6 measure the relative flag leaf greenness after the fungicide/foliar N fertilizer
7 application. HNT is a hand-held instrument that measures the light transmitted by a
8 plant leaf at two different wavelengths (650 and 960 nm) (Arregui *et al.*, 2006). The
9 ratio of the light transmitted at these wavelengths, in addition to the ratio determined
10 with no sample, is processed by the instrument to produce a digital reading. The HNT
11 values are numerical, dimensionless values that are proportional to the amount of
12 total chlorophyll present in the leaf.

13 Readings were taken using the HNT at midlength of the flag leaf from 30 randomly
14 selected plants per plot. The HNT measurements were carried out at different growth
15 stages: end of stem elongation, with the flag leaf just visible (GS 37), heading (GS
16 59), early dough (GS 83) and hard dough (GS 87). This last stage was used to
17 distinguish between plots in which the flag leaves were still photosynthesizing and
18 those in which nitrogen translocation to grains was almost complete.

19

20 Grain protein, ash content and vitreousness

21 Grain samples from each plot were milled into white flour using a Brabender
22 Quadrumat Junior Mill (Brabender, Duisberg, Germany), fitted with a 200 µm
23 aperture sieve. Grain protein and ash content were determined by near-infrared

1 reflectance spectroscopy, using a NIRSystems 6500 monochromator instrument
2 (Foss-NIRSystems, Silver Spring, MD, USA) and presented on a dry matter basis.
3 The vitreousness was determined as percentage of vitreous kernels according to the
4 method given in ICC Standard 129, with a farinator, a device that allows 50 wheat
5 kernels to be held firmly while a blade cuts them transversely. The vitreousness was
6 determined by examining the cross-section of 100 cut kernels. Vitreous grain appear
7 dark and translucent, while opaque grains appear yellow and translucent.

8

9 FHB symptoms and DON analyses

10 FHB incidence and severity were recorded for each plot, carrying out visual
11 evaluations of the disease at the soft dough stage (GS 85).

12 FHB head blight incidence was calculated as the percentage of plants with symptoms
13 that were recorded when 200 ears per plot were analysed.

14 FHB severity was computed as the percentage of kernels per ear with symptoms. A
15 scale of 1 to 7 was used in which each numerical value corresponds to a percentage
16 interval of surfaces exhibiting visible symptoms of the disease according to the
17 following schedule: 1 = 0-5%, 2 = 5-15 %, 3 = 15-30%; 4 = 30-50 %, 5 = 50-75%, 6 =
18 75-90%, 7 = 90-100% (Parry *et al.*, 1995). The FHB severity scores were converted
19 to percentages of the ear exhibiting symptoms, replacing each score with the mid-
20 point of the interval.

21 A 2 kg representative sample of grain from each plot was freeze-dried and milled. 25
22 g representative sub-samples of the milled material were extracted by shaking for at
23 least 30 min with 100 ml of MilliQ water containing 5 g of polyethylene glycol 8000.
24 The supernatant was filtered through filter paper (0.45 µm) and cleaned using a

1 Donprep column (R-Biopharm® Rhone LTD) by rinsing with 5 ml of MilliQ water. The
2 DON was then eluted using 1.5 ml of methanol and 250 µl of water/acetonitrile 90/10.
3 A 25 µl solution was injected into an HPLC column (Synergi 4µ Fusion-RP80A
4 Phenomenex column, 1ml min⁻¹; detector DAD-UV, 220-225 nm). Toxin quantification
5 was performed using external standards and peak height measurements. The
6 detection limit was 10 µg kg⁻¹.

7

8 Statistical analysis

9 The normal distribution and homogeneity of variances were verified by performing
10 the Kolmogorov–Smirnov normality test and the Levene test, respectively.

11 An analysis of variance (ANOVA) was utilized to compare the HNT readings, STB
12 severity, FHB incidence and severity, DON content, grain yield, test weight, grain
13 protein content, ash content and vitreousness using a completely randomized block
14 design, in which the treatment was the independent variable. Multiple comparison
15 tests were performed according to the SNK test on treatment means. The statistical
16 package SPSS for Windows, Version 13.0 (SPSS Inc., Chicago) was used for the
17 statistical analysis.

18 The STB severity, FHB incidence and severity values were arcsin square root
19 transformed before further statistical analysis, as percentage data derived from
20 counting.

1 **Results**

2 **Weather conditions**

3 The two growing seasons showed different meteorological trends from the beginning
4 of the stem elongation stage to harvest (Tab. 3): in 2007, during the stem elongation
5 phase there was very little rainfall, while frequent rainfall occurred at anthesis and at
6 the end of ripening, after the soft dough stage, but the last rainfall was late and was
7 not able to prolong grain filling duration. In 2008, instead, the precipitations were
8 frequent and regular from April to June, above all from the beginning of flowering to
9 the soft dough stage, prolonging the harvest till the end of the first and second
10 decade of July.

11

12 **STB severity and flag leaf greenness**

13 The severity of STB symptoms recorded during the visual evaluations of flag leaf was
14 higher in the 2007-2008 than in the to 2006-2007 growing season (table 4). In all the
15 experiments, ANOVA showed a significant effect of fungicide treatment on STB
16 severity. The application of a azole-only programme at heading (T2) reduced the
17 STB severity compared to the untreated control (T1) at S2, S3 and S4 in the 2007-
18 2008 growing season. Considering the average data of all the experiments, a
19 strobilurin fungicide applied at the stem elongation stage and azole fungicide applied
20 at heading (T3) showed a significantly lower STB severity (35% lower) than the
21 untreated control. In all the experiments, with the exception of S3 in the 2006-07
22 growing season, the T3 treatment had a significantly lower STB severity than the T2

1 treatment (19% lower). The strobilurin or foliar N fertilizer addition at heading only
2 significantly affected the STB control in the 2006-2007 period at S3.

3 Flag leaf damage from other diseases, such as powdery mildew and rusts, was
4 generally low, in particular in 2007.

5 The HNT reading clearly described the greenness status of the flag leaves during
6 different GSs (table 4). In each growing season and at each site, the HNT flag leaf
7 values increased from GS 37 to GS 59, and then a reduction was observed. The
8 decrease in HNT readings was particularly evident from GS 83 to GS 87.

9 No significant differences were observed for the HNT values of the flag leaves at GS
10 37 or GS 59. At GS 83, ANOVA showed a significant effect of foliar treatment on the
11 HNT values in the 2 experiment conducted in 2006-2007: significant differences were
12 observed between treatment T5 (a foliar N fertilizer in addition to a azole-strobilurin
13 programme) and T1 (untreated control). In the 2007-2008 growing season, a
14 significant effect ($P < 0.001$) at GS 83 was only observed at S1: the azole fungicide
15 application significantly increased the HNT values of flag leaf (by 10%) compared to
16 the untreated control. No significant differences were observed for the application of
17 strobilurin fungicide at stem elongation or at the heading stage or with the foliar N
18 fertilizer application.

19 ANOVA showed always a significant effect of foliar treatment on the HNT values at
20 GS 87. The azole fungicide application at heading (T2) significantly delayed
21 senescence of the flag leaf compared to the untreated control (T1) in 2006-2007 at
22 S3 and in 2007-2008 at S2, S3 and S4. In 2007-2008 growing season, at S2 and S4,
23 a significant further increase in HNT values was observed with the application of
24 strobilurin fungicide at stem elongation (T3). In all the experiments the T5 treatment
25 showed higher HNT values than the untreated control (T1). A significant effect of

1 strobilurin fungicide application at heading on delaying senescence of the flag leaf
2 was observed in 2006-2007 at S2, while no significant differences were observed in
3 any experiments between the azole application at heading (T3) and the addition of a
4 foliar N fertilizer (T4).

5

6 FHB symptoms and DON contamination

7 The incidence of FHB symptoms recorded during the visual evaluations were similar
8 in all the growing seasons and sites, while the FHB severity values were generally
9 higher in the experiment conducted in 2007-2008 than those performed in the
10 previous growing season (table 5).

11 ANOVA showed a significant effect of foliar treatment on FHB incidence and severity
12 in all the experiments, except for the FHB incidence in both growing seasons at S3.

13 The azole application at heading (T2) reduced the FHB incidence and severity
14 compared to the untreated control (T1), with a reduction of 14% and 11% for the two
15 parameters, respectively.

16 The strobilurin or foliar N fertilizer addition did not generally affect the FHB control
17 compared to the azole-only control; only at S4 in 2007-2008 was the FHB severity in
18 T4 and T5 significantly lower (by 8%) compared to T2.

19 The DON contamination was generally higher in grain samples harvested in 2008
20 than those of 2007. In all the experiments, a significant effect of the azole application
21 was observed at heading (T2) on DON occurrence. The contamination of the T2
22 treatment was 51% lower than the T1 one. The addition of a foliar N fertilizer or the
23 application of a strobilurin fungicide at the stem elongation stage did not show any
24 significant differences for DON content at harvest compared to the azole programme

1 at heading. On the other hand, the strobilurin fungicide application at heading led to a
2 higher DON occurrence (36% higher) in grains harvested at S2 and at S4 in 2007-
3 2008 compared to the T3 treatment. No significant differences were observed
4 between this treatment (T5) and the untreated control (T1) in any of the experiments
5 conducted in this growing season.

6

7 Yield, test weight, grain protein, gluten and ash content

8 ANOVA showed a significant effect of foliar treatment on grain yield in all the
9 experiments (table 6). With the exception of the experiment conducted at S3 in 2007-
10 2008, the azole application at heading (T2) significantly increased yield compared to
11 the untreated control (T1), with an increase of 22%. A significant further increase in
12 grain yield was observed at S1 and S3 in 2007-2008 for the strobilurin application at
13 the stem elongation stage (T3). The addition of a foliar N fertilizer or a strobilurin
14 fungicide at heading to a azole programme did not lead to any significant increase in
15 the grain yield compared to T3.

16 The test weight was generally lower in the grain samples harvested in 2008 than
17 those of 2007, as a consequences of the higher FHB and foliar disease pressure.

18 ANOVA showed significant differences between the treatments concerning the test
19 weight in the experiments conducted at S2 in 2006-2007 and at S1, S3 and S4 in the
20 2007-2008 growing season. At S1 and S4 (2007-2008), the azole application at
21 heading (T2) significantly increase, the test weight by 7% compared to the untreated
22 control (T1). The test weight was significantly higher at S2 in 2006-2007 and at S1
23 and S3 in 2007-2008 when a strobilurin fungicide, applied at stem elongation (T3),
24 was added to a azole programme at heading (T2). The application of a nitrogen foliar

1 fertilizer or a strobilurin in addition at heading did not lead to any significant increase
2 in test weight compared to T3 in any experiments.

3 The average protein content of the durum wheat used in the trials was 17.6% in
4 2006-2007 and 15.7% in 2007-2008, which was appropriate for semolina production
5 according to industry standards (Clarke, 2001). ANOVA, only showed a significant
6 effect of the foliar treatment on the grain protein content at S3 in the 2007-2008
7 experiment: the N foliar fertilizer and strobilurin addition to a azole application at
8 heading (T5) showed a higher protein content than treatment T3, with only the azole
9 application at heading.

10 No significant differences were observed for the ash content and vitreousness among
11 the treatments in any experiment.

1 **Discussion**

2 The data of this research underline that the cultivation of durum wheat in northern
3 Italy can lead to considerably high grain quality, in terms of protein and gluten
4 content. The observed grain protein content was always higher than 15%, with a
5 value that, in certain cases, arrived at 18%. The grain protein content has been
6 confirmed to be influenced by climatic parameters, soil fertility and available moisture
7 during grain filling (Rharrabti *et al.*, 2003b).

8 The susceptibility of this crop to STB and FHB has also been confirmed as a
9 consequence of climatic conditions in spring which were favourable to fungal disease
10 development in both years. Although the grain yield and test weight obtained in the
11 2006-2007 growing season were quite good, the higher foliar disease and FHB
12 pressure of 2007-2008 growing season led to poor yield and test weight. Moreover,
13 the grains harvested in the 2007-2008 showed a generally higher DON content than
14 the admissible maximum levels ($1750 \mu\text{g kg}^{-1}$) based on UE regulations (EC, 2006).
15 These data clearly show that the cultivation of durum wheat in the climatic conditions
16 of North Italy is at present risky and that a direct control of fungal disease, which is
17 able to reduce the development of both foliar and head attacks, is necessary in the
18 years with climatic conditions favourable to their development.

19 The azole application at heading led to a clearly higher grain yield, lower FHB
20 symptoms and a lower DON contamination, in years as well high as low disease
21 pressure. The increase in grain yield that was observed was mainly due to an
22 incidence of shrivelled kernels. The results obtained for these fungicide treatments
23 for FHB and DON control are in agreement with those obtained on common wheat by
24 Koch *et al.* (2006) in Germany and by Blandino *et al.* (2006) in North Italy and on

1 durum wheat by Menniti et al. (2003). Azoles have been confirmed to be effective
2 against FHB infection and DON contamination, (Haidukowski *et al.*, 2005) although in
3 the second year of this research, with conditions of higher disease pressure, their
4 application was not able to achieve a sufficient level of control of FHB. The cultivation
5 of durum wheat in northern Italy therefore needs to take in account all the other
6 agricultural practices that are able to minimize foliar disease damage and DON
7 contamination risk in wheat grains. Precautionary measures to control STB and FHB
8 infection should be taken into account at the beginning of wheat cultivation,
9 especially in the regions more prone to fungal attacks, are avoiding cereal
10 succession, incorporating the previous crop residues and using a resistant cultivar
11 (Pirgozliev *et al.*, 2003).

12 Only with a higher STB pressure, was the azole treatment at heading able to reduce
13 this foliar disease severity and to delay the senescence of flag leaves, compared to
14 the untreated control, confirming a partial action of these fungicides on controlling
15 *Septoria tritici* and other foliar disease (Mavroeidi and Shaw, 2006). Furthermore, the
16 control of STB was significantly reduced by the strobilurin application at the stem
17 elongation stage. This treatment led to higher leaf greenness in the flag leaves and a
18 further increase in grain yield, particularly in the experiment with higher STB
19 pressure.

20 As expected, leaf greenness was influenced by fungicide, especially by the strobilurin
21 application at stem elongation. These data confirms that delaying the senescence of
22 flag leaves reduces the decline in physiological activity and could assure higher grain
23 yields (Ruske et al., 2003a). Chlorophyll meters, such as HNT, have been confirmed
24 to be useful instruments to describe the greenness status of flag leaves and they
25 could predict differences in wheat yield, as reported by Vidal *et al.* (1999). The

1 double strobilurin application (at the stem elongation stage and at heading) did not
2 show any significant advantage on the control of the main foliar diseases, particularly
3 STB, and, consequently, on grain yield, compared to the single application at the
4 stem elongation stage. Moreover, according to Dimmock and Gooding (2002a), the
5 addition of strobilurin to an azole programme at heading did not significantly delay
6 senescence of the flag leaves, compared to the azole-only application. On the other
7 hand, the application of this fungicide at heading led to an increase in DON contents
8 that was often not significantly different from that of the untreated controls, confirming
9 data collected in the same area in a previous trial on common wheat (Blandino *et al.*,
10 2006). As suggested by Pirgozliev *et al.* (2003), the increase in DON observed with
11 the strobilurin application is probably due to an increase in the infection of the
12 *Fusarium* species, following the reduction of the presence of *Microdochium nivale*, a
13 pathogen which, unlike other *Fusarium* species, is involved in the symptomatology of
14 the disease, but which is not able to synthesise DON.

15 No effect of fungicide application on the protein content, ash concentration or
16 vitreousness was recorded. The impact of STB control on the grain quality of
17 common wheat has not been understood clearly either. Several authors (Peltonen
18 and Karjalainen, 1992; Dimmock and Gooding, 2002b) have reported that the control
19 of this foliar disease resulted in an increased wheat grain protein content, because of
20 the prolonged duration of the green leaf area and the grain filling phase. Ruske *et al.*
21 (2001) instead showed that protein concentrations were reduced significantly
22 following *Septoria tritici* control. No significant effect of foliar fungicide application on
23 grain protein was observed in other studies (Kelley *et al.*, 1993; McKendry *et al.*,
24 1995). Several studies have reported that if the crop is kept healthy, the
25 photosynthetic period is prolonged and more of the nitrogen taken up by the plant

1 can be translocated to the grain (Dimmok and Gooding, 2002a). On the other hand,
2 conditions that promote leaf senescence during grain filling, such as foliar disease,
3 tend to increase protein deposition over starch accumulation in the grain, because
4 the production and translocation of carbohydrates to the grain is more sensitive to
5 adverse growing conditions than protein production (Fernandez- Figares *et al.*,
6 2000). Probably both these effects played a role in this study, thus no significant
7 effect of disease control on grain protein content was observed.

8 At the grain yield and quality levels reached in these experiments, the foliar N
9 fertilizer applications had no effects on the grain yield and had an almost negligible
10 effect on the grain protein content. This was also observed in Spain by Abad *et al.*
11 (2004) and Garrido-Lestache *et al.* (2005). On the other hand, Ottman *et al.* (2000)
12 reported that foliar application of N, in the form of urea, at ear emergence increased
13 grain protein concentration, but did not affect grain yield or other quality indices. The
14 results of several studies on common wheat have shown that yield responses to
15 foliar N fertilizer are highly variable and yield is only increased when the previous N
16 applications to the soil had been sub-optimal (Gooding and Davies, 1992; Readman
17 *et al.*, 1997). This suggests that the rates of mineral N used in the experiments and
18 the fertility of the soils were too high to optimize the use of late foliar fertilizer at the
19 production or quality levels obtained.

20 In contrast to the data reported by Garrido-Lestache *et al.* (2005), foliar application of
21 N at heading in the present study did not increase the grain ash content.

22 In conclusion, the experiments conducted in 2 growing seasons have confirmed the
23 criticality of the disease management of this crop in North Italy, especially in years
24 with climatic conditions that are favourable to fungal pathogen development. Thus,
25 the diffusion of this cereal to achieve high quality durum chains, in areas with

1 weather conditions similar to those of the present study, could only be effective with a
2 successful integrated programme to control foliar disease, FHB and DON
3 contamination. At the moment, among the applied control methods, fungicide
4 treatment plays an important and crucial but not decisive role. The double treatment,
5 with a strobilurin application during the stem elongation stage and azole at heading,
6 has emerged as an essential practice. Other foliar treatments at heading, such as
7 strobilurin or foliar N fertilizer applications, did not seem to provide any further
8 advantage, in either grain yield or quality. However, the application of strobilurin
9 fungicide at heading could be a risky practice, since it could reduce the efficacy of
10 azole fungicide to control DON contamination.

11 Although other studies are necessary to verify the effect of foliar treatment
12 programmes on durum wheat in areas more prone to these fungal disease, and to
13 check other and new fungicides, the cultivation of this crop in these regions could
14 only be possible with the development and the selection of varieties which show a
15 higher resistance to STB and FHB and the adoption of all the other preventive
16 agricultural practices that are able to minimize their attacks.

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Tables

Table 1

Treatments compared in the experimental fields conducted at 2 sites in the 2006-2007 period and at 4 sites in the 2007-2008 period in North Italy.

Treatment	Fungicide application		foliar N application
	stem elongation (GS 35)	heading (GS 59)	heading (GS 59)
T1	-	-	-
T2	-	prochloraz + ciproconazole ^a	-
T3	azoxystrobin ^b	prochloraz + ciproconazole ^a	-
T4	azoxystrobin ^b	prochloraz + ciproconazole ^a	foliar N fertilizer ^c
T5	azoxystrobin ^b	prochloraz + ciproconazole ^a + azoxystrobin ^b	foliar N fertilizer ^c

^a a.i. prochloraz + cyproconazole (Tiptor[®] S, Syngenta Crop Protection) were applied at 0.36 and 0.048 kg (a.i.) ha⁻¹, respectively.

^b a.i. azoxystrobin (Amistar[®], Syngenta Crop protection) was applied at 0.25 kg (a.i.) ha⁻¹.

^c The foliar nitrogen fertilizer applied was YaraVita[™] Last[®] N at 4.68 kg N ha⁻¹.

GS growth stage following the Zadoks scale (Zadoks et al., 1974).

Table. 2

Main trial information for the field experiments conducted in the 2006-2008 period in North Italy.

Growing seasons	Sites	Sowing date	Date of fungicide/foliar N application		Harvest date	Previous crop
			rising	heading		
2006-2007	S2	October 25, 2006	March 11, 2007	May 3, 2007	June 29, 2007	maize
	S3	October 30, 2006	March 14, 2007	May 4, 2007	June 26, 2007	soft wheat
2007/2008	S1	November 5, 2007	April 6, 2008	May 12, 2008	July 2, 2008	maize
	S2	November 7, 2007	April 4, 2008	May 10, 2008	July 18, 2008	durum wheat
	S3	November 15, 2007	April 8, 2008	May 15, 2008	July 17, 2008	soft wheat
	S4	November 3, 2007	April 4, 2008	May 13, 2008	July 9, 2008	maize

Table. 3

Rainfall and rainy days from the end of tillering to harvesting in the 2006-2008 period at the research sites.

Year	2007				2008							
	S2		S3		S1		S2		S3		S4	
Site	Rainfall	Rainy days	Rainfall	Rainy days	Rainfall	Rainy days	Rainfall	Rainy days	Rainfall	Rainy days	Rainfall	Rainy days
GS*	(mm)	(d)	(mm)	(d)	(mm)	(d)	(mm)	(d)	(mm)	(d)	(mm)	(d)
29-59	49	13	41	14	74	16	86	16	95	18	101	15
61-69	61	11	56	9	55	10	51	10	69	13	56	10
71-85	49	11	62	11	87	16	93	17	69	13	135	16
86-99	106	22	111	20	47	11	72	12	93	12	51	11
29-99	266	57	270	54	263	53	302	55	327	56	343	52

(*) growth stage (Zadoks *et al.*, 1974)

Table. 4

Effect of fungicide and foliar N applications on STB severity on flag leaf and N tester readings at different growth stages; field experiments conducted in North Italy in the 2006-2008 period.

Growing seasons	Site	Treatment ^a	STB severity ^b		HNT values (HNT unit)			
			T	N (%)	GS 37	GS 59	GS 83	GS 87
2006-2007	S2	T1	25.2 a	18.3	646 a	705 a	577 b	406 b
		T2	22.3 a	14.4	636 a	696 a	590 ab	391 b
		T3	17.0 b	8.7	636 a	719 a	600 ab	360 b
		T4	14.5 b	6.4	643 a	707 a	625 ab	406 b
		T5	14.3 b	6.1	624 a	711 a	663 a	611 a
		P (F)	< 0.001		0.745	0.639	0.044	0.001
	S3	T1	21.7 a	13.8	746 a	809 a	722 b	40 a
		T2	19.1 a	10.8	747 a	805 a	782 ab	137 a
		T3	18.0 a	9.7	739 a	800 a	791 ab	146 a
		T4	14.0 b	6.0	747 a	805 a	844 a	131 a
		T5	12.2 b	4.6	766 a	809 a	849 a	150 a
P (F)		< 0.001		0.473	0.978	0.031	0.129	
2007-2008	S1	T1	46.1 a	52.0	558 a	703 a	517 c	326 c
		T2	42.9 a	46.3	549 a	704 a	611 b	373 bc
		T3	34.3 b	32.0	556 a	701 a	611 b	416 abc
		T4	30.7 b	26.4	558 a	712 a	638 a	442 ab
		T5	31.3 b	27.7	552 a	706 a	640 a	484 a
		P (F)	0.004		0.991	0.959	< 0.001	0.008
	S2	T1	35.2 a	33.6	538 a	668 a	672 a	335 c
		T2	28.3 b	22.6	528 a	677 a	694 a	401 b
		T3	25.5 b	16.1	544 a	681 a	705 a	470 a
		T4	22.7 b	14.9	566 a	675 a	703 a	471 a
		T5	22.9 b	15.3	539 a	687 a	706 a	492 a
		P (F)	< 0.001		0.889	0.929	0.371	0.001
	S3	T1	41.9 a	44.7	677 a	654 a	465 a	270 c
		T2	31.4 b	27.3	679 a	666 a	487 a	336 b
		T3	26.1 c	19.4	677 a	670 a	554 a	373 ab
		T4	25.0 c	17.9	668 a	678 a	549 a	383 ab
		T5	24.5 c	17.3	675 a	663 a	565 a	409 a
P (F)		< 0.001		0.995	0.858	0.178	0.001	
S4	T1	34.4 a	32.7	632 a	671 a	675 a	400 c	
	T2	27.8 b	21.9	623 a	688 a	712 a	498 b	
	T3	22.9 c	15.1	625 a	697 a	715 a	571 a	
	T4	23.8 c	16.3	622 a	679 a	711 a	575 a	
	T5	22.9 c	15.2	624 a	687 a	715 a	588 a	
	P (F)	0.001		0.995	0.517	0.137	< 0.001	
Average data ^c	T1	34.8 a	32.5	633 a	708 a	607 b	296 b	
	T2	27.8 b	23.9	627 a	706 a	654 ab	356 ab	
	T3	22.9 c	16.8	629 a	711 a	661 ab	389 ab	
	T4	23.8 c	14.6	634 a	709 a	669 ab	401 ab	
	T5	22.9 c	14.3	630 a	710 a	690 a	456 a	
	P (F)	< 0.001		0.998	0.997	0.045	0.004	

^a Treatment: see table 1. Means followed by different letters are significantly different (the level of significance is shown in the table). Reported values are based on 4 replications.

^b STB severity was calculated as the percentage of flag leaves with symptoms of disease at the soft dough stages (GS 85). Means reported are values transformed (T; $y' = \arcsin \sqrt{x} * 180/\pi$) and not transformed (N).

^c Average data of 6 experiments conducted in 2007-08 and 2008-09 growing seasons.

Table. 5

Effect of fungicide and foliar N applications on FHB incidence and severity and DON contamination in the durum wheat kernels; field experiments conducted in North Italy in the 2006-2008 period.

Growing seasons	Site	Treatment ^a	FHB incidence ^c		FHB severity ^d		DON ($\mu\text{g kg}^{-1}$)	
			T	N (%)	T	N (%)		
2006-2007	S2	T1	78.8 a	94.8	18.7 a	10.4	1363 a	
		T2	61.0 b	76.1	10.3 b	3.3	690 b	
		T3	60.2 b	72.7	10.2 b	3.5	571 b	
		T4	58.5 b	72.2	8.6 b	2.3	654 b	
		T5	62.5 b	78.7	11.0 b	3.9	716 b	
		P (F)	0.026		0.002		< 0.001	
	S3	T1	73.1 a	91.3	13.2 a	5.3	40 a	
		T2	53.9 a	64.7	8.5 b	2.3	nd b	
		T3	61.3 a	75.2	8.8 b	2.4	nd b	
		T4	56.6 a	68.8	8.3 b	2.1	nd b	
		T5	53.7 a	61.1	8.1 b	2.3	nd b	
		P (F)	0.339		0.038		< 0.001	
	2007-2008	S1	T1	68.6 a	84.8	24.6 a	17.8	3647 a
			T2	57.4 abc	70.6	13.8 b	6.0	2642 b
			T3	63.1 ab	79.1	16.4 b	8.5	3077 ab
T4			51.5 bc	61.2	10.3 b	3.2	3002 ab	
T5			48.2 c	55.6	9.5 b	2.8	3400 ab	
P (F)			0.003		< 0.001		0.037	
S2		T1	75.6 a	93.7	32.6 a	29.0	5230 a	
		T2	56.4 b	68.8	13.7 b	5.6	2697 b	
		T3	52.3 b	62.5	14.8 b	6.6	2512 b	
		T4	54.6 b	65.4	15.1 b	7.3	3770 ab	
		T5	59.6 b	73.8	14.8 b	6.6	4907 a	
		P (F)	0.002		< 0.001		0.009	
S3		T1	83.8 a	97.7	41.1 a	43.3	1400 a	
		T2	79.8 a	93.9	28.8 b	23.7	703 a	
		T3	79.3 a	95.3	31.6 b	27.6	930 a	
	T4	74.7 a	92.8	29.0 b	23.5	852 a		
	T5	74.7 a	92.5	29.6 b	24.4	1002 a		
	P (F)	0.435		0.002		0.263		
S4	T1	85.3 a	98.7	35.9 a	34.4	2697 a		
	T2	70.8 b	88.9	25.2 b	18.1	1605 b		
	T3	76.8 ab	93.1	22.9 b	15.1	1933 b		
	T4	64.4 b	80.8	16.8 c	8.5	1792 b		
	T5	62.7 b	77.7	17.4 c	9.5	2215 ab		
	P (F)	0.003		< 0.001		0.005		
Average data ^d	T1	77.5 a	93.5	27.7 a	23.4	2396 a		
	T2	63.2 b	77.2	16.7 b	9.8	1390 b		
	T3	65.5 b	79.6	17.4 b	10.6	1504 b		
	T4	60.1 b	73.5	14.7 b	7.8	1679 b		
	T5	60.2 b	73.2	15.0 b	8.2	2040 ab		
	P (F)	< 0.001		< 0.001		0.029		

^a Treatment: see table 1. Means followed by different letters are significantly different (the level of

significance is shown in the table). Reported values are based on 4 replications. FHB incidence and severity means reported are values transformed (T; $y' = \arcsin \sqrt{x} * 180/\pi$) and not transformed (N).

^b FHB incidence was calculated as the percentage of ears with symptoms of disease at the soft dough stages (GS 85).

^c FHB severity was calculated as the percentage of kernels per ear with symptoms of disease at the soft dough stages (GS 85).

nd: not detected. The detection limit was $10 \mu\text{g kg}^{-1}$.

^d Average data of 6 experiments conducted in 2007-08 and 2008-09 growing seasons.

1 **Table. 6**

2 Effect of fungicide and foliar N applications on grain yield, test weight, grain protein
 3 content, ash content and vitreousness in the durum wheat kernels; field experiments
 4 conducted in North Italy in the 2006-2008 period.

Growing seasons	Site	Treatment ^a	Yield t ha ⁻¹	Test weight g	Protein (%)	Ash (%)	Vitreousness (%)
2006-2007	S2	T1	4.8 b	72.7 a	18.6 a	2.4 a	90.4 a
		T2	5.5 a	73.9 a	18.1 a	2.3 a	92.8 a
		T3	5.9 a	74.6 a	18.5 a	2.3 a	91.8 a
		T4	5.6 a	73.7 a	17.8 a	2.3 a	91.5 a
		T5	5.8 a	74.6 a	18.0 a	2.3 a	92.6 a
		<i>P</i> (F)	0.005	0.100	0.739	0.900	0.689
	S3	T1	4.6 b	71.5 a	17.0 a	2.1 a	90.8 a
		T2	5.4 a	73.5 a	16.8 a	2.2 a	89.7 a
		T3	5.5 a	73.4 a	17.0 a	2.1 a	90.8 a
		T4	5.6 a	73.7 a	17.0 a	2.2 a	89.7 a
		T5	5.6 a	73.6 a	17.4 a	2.2 a	91.7 a
		<i>P</i> (F)	0.018	0.609	0.990	0.758	0.984
2007-2008	S1	T1	3.1 c	60.3 c	16.3 a	2.0 a	84.5 a
		T2	3.8 b	63.2 b	15.6 a	2.0 a	85.4 a
		T3	4.3 a	65.9 a	15.8 a	2.0 a	84.0 a
		T4	4.5 a	66.9 a	15.7 a	2.0 a	87.0 a
		T5	4.4 a	66.4 a	16.1 a	1.9 a	86.3 a
		<i>P</i> (F)	< 0.001	< 0.001	0.290	0.922	0.753
	S2	T1	3.2 c	66.9 a	16.0 a	1.7 a	85.9 a
		T2	3.7 b	67.8 a	15.4 a	1.7 a	87.6 a
		T3	3.8 ab	68.0 a	15.7 a	1.8 a	87.2 a
		T4	3.9 ab	68.5 a	15.6 a	1.7 a	87.6 a
		T5	4.1 a	68.6 a	16.0 a	1.7 a	88.5 a
		<i>P</i> (F)	< 0.001	0.184	0.155	0.520	0.637
	S3	T1	2.5 b	59.9 b	15.0 ab	1.8 a	84.8 a
		T2	2.7 b	61.8 b	14.9 ab	1.8 a	84.3 a
		T3	3.6 a	67.1 a	14.4 b	1.8 a	85.8 a
		T4	3.7 a	66.0 a	14.9 ab	1.8 a	85.9 a
		T5	3.9 a	65.7 a	15.1 a	1.8 a	84.5 a
		<i>P</i> (F)	0.001	< 0.001	0.041	0.454	0.827
	S4	T1	1.9 b	54.6 b	16.3 a	2.0 a	84.0 a
		T2	2.7 a	59.2 a	16.1 a	1.8 a	83.7 a
		T3	2.8 a	59.7 a	15.9 a	2.0 a	85.3 a
		T4	2.7 a	59.2 a	16.2 a	1.9 a	85.3 a
		T5	2.9 a	59.6 a	16.3 a	1.8 a	86.2 a
		<i>P</i> (F)	0.001	0.003	0.782	0.090	0.555
Average data ^b		T1	3.4 b	64.3 a	16.5 a	2.0 a	86.8 a
		T2	4.0 ab	66.6 a	16.2 a	2.0 a	87.3 a
		T3	4.4 a	68.1 a	16.3 a	2.0 a	87.5 a
		T4	4.4 a	68.0 a	16.2 a	2.0 a	87.8 a
		T5	4.4 a	68.1 a	16.3 a	2.0 a	88.0 a
		<i>P</i> (F)	0.008	0.111	0.866	0.976	0.810

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6 ^a Treatment: see table 1. Means followed by different letters are significantly different (the level of

- 1 significance is shown in the table). Reported values are based on 4 replications.
- 2 ^b Average data of 6 experiments conducted in 2