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

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Abstract

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

Field experiments have been conducted over two growing seasons at four sites in North West Italy to evaluate the effect of fungicides and foliar nitrogen fertilizer application on durum wheat yield and grain quality. Five combinations of foliar application were compared at each site and each year (untreated control, azole fungicide application at heading, strobilurin fungicide at the stem elongation stage and/or at heading, the addition of a foliar N fertilizer to a fungicide programme). The following parameters were analyzed: Septoria Tritici Blotch (STB) severity, flag leaf greenness using a chlorophyll meter, grain yield, test weight, grain protein content, ash content, vitreousness, Fusarium head blight (FHB) incidence and severity and deoxynivalenol (DON) contamination. The collected data underline that the cultivation of durum wheat at the climatic conditions of North Italy is actually risky and needs a direct control of fungal disease, which would be able to reduce the development of both foliar and head attacks. The double treatment, with a strobilurin application during the stem elongation stage and azole at heading, results to be an essential practice and showed advantages in terms of the delay of flag leaf senescence (+27%), STB control (+31), FHB control (+11%), yield (+32%) and DON contamination (-45%), compared to the untreated control. Other foliar treatments at heading, such as strobilurin or foliar N fertilizer applications, do not seem to provide any further advantage, for either grain yield or quality. No significantly effect of fungicide or foliar N fertilizer application was recorded on the protein or ahs concentration or vitreousness.

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

Introduction

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

In recent years, improving durum wheat grain quality has become, one of the main breeding and agronomic goals in many Mediterranean countries, due to the increase in market demand for good quality grains (De Vita *et al*., 2007). The protein content and gluten quality have long been recognized as the most important factors that can affect pasta-making properties (Kovacs *et al*., 1997). Since production in South Italy is characterized by high variability, in terms of yield and grain quality, mainly because it is affected by the severity and irregularity of water stress and high temperatures (Dunkeloh and Jacobeit, 2003), there is also considerable interest in durum wheat cultivation in more temperate regions, such as northern Italy, where the climatic conditions during grain filling could offer the possibility of obtaining grain yield with a higher technological quality (Rharrabti *et al*. 2003a). In general, the cultivation of durum wheat in cooler regions could favour heavy and more vitreous grains, with a considerable increase in grain protein and gluten content (Gooding *et al*., 2003), but the grain quality could be negatively influenced by high percentages of ash accumulated in the kernels (Rharrabti *et al*., 2003a). On the other hand, cooler and wetter weather conditions make durum wheat more prone to fungal disease (Pascale *et al*., 2002), particularly to Septoria Tritici Blotch (STB) and Fusarium Head Blight (FHB).

STB, caused mainly by *Septoria tritici* Rob., has been associated with yield losses, due to the reduction of the photosynthetic life of the canopy, especially in the flag leaf, during grain filling (Puppala *et al*., 1998). Moreover, both STB and FHB reduce the yield and grain quality by causing shrivelled kernels and reduced test weight (McKendry *et al*., 1995; Parry *et al*., 1995). *Fusarium graminearum* and *F. culmorum*, the most important agents of FHB, are also the main causes of the accumulation, in wheat kernels, of deoxynivalenol (DON), a mycotoxin of the trichotecenes group, which is associated with serious mycotoxicosis in humans and animals (Bottalico and Perrone, 2002). Pascale *et al*. (2002) observed that durum wheat varieties are generally more prone to DON contamination than common wheat, particularly when cultivated in more temperate regions. Since no durable, fully resistant wheat cultivars exist at present (Snijders, 2004), the main strategy to protect durum wheat from these foliar and head diseases is through fungicide applications, both during the stem elongation stage and at anthesis (Ruske *et al*., 2003b). Among the various fungicides that are available, azole applications at anthesis have resulted to have a significant effect on the reduction of the decline of green leaf area in flag leaves (Kettlewell *et al*., 1982) and on the increase of grain yield (Matthies and Buchenauer, 2000). Fungicides containing triazole (the most important are bromuconazole, epoxiconazole, metconazole, propioconazole, tebuconazole and tetraconazole), imidazole (prochloraz) or triazolinthione (prothioconazole) active ingredients which inhibit the biosynthesis of ergosterol, have proved to be the most active molecules against FHB infection and DON contamination (Menniti et al., 2003; Paul et al., 2008).

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

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

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

Materials and Methods

Experimental site and treatments

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

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

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

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

The following active ingredients or products were used:

- Azole fungicide: mixture of prochloraz and cyproconazole (Tiptor® S, Syngenta Crop Protection, Italy, formulation: emulsifiable concentrate (EC), applied at 0.36 and 0.048 kg active ingredient (a.i.) ha^{-1}, respectively;
- Strobilurin fungicide: azoxystrobin (Amistar®, Syngenta Crop Protection, Italy, formulation: suspension concentrate), applied 0.25 kg active ingredient (a.i.) ha^{-1};

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

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

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

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

STB severity and flag leaf greenness

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

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

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

Grain protein, ash content and vitreousness

Grain samples from each plot were milled into white flour using a Brabender Quadrumat Junior Mill (Brabender, Duisberg, Germany), fitted with a 200 μm aperture sieve. Grain protein and ash content were determined by near-infrared reflectance spectroscopy, using a NIRSystems 6500 monochromator instrument (Foss-NIRSystems, Silver Spring, MD, USA) and presented on a dry matter basis.

The vitreousness was determined as percentage of vitreous kernels according to the method given in ICC Standard 129, with a farinator, a device that allows 50 wheat kernels to be held firmly while a blade cuts them transversely. The vitreousness was determined by examining the cross-section of 100 cut kernels. Vitreous grain appear dark and translucent, while opaque grains appear yellow and translucent.

FHB symptoms and DON analyses

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

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

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

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

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

Statistical analysis

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

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

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

Results

Weather conditions

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

STB severity and flag leaf greenness

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

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

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

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

ANOVA showed always a significant effect of foliar treatment on the HNT values at GS 87. The azole fungicide application at heading (T2) significantly delayed senescence of the flag leaf compared to the untreated control (T1) in 2006-2007 at S3 and in 2007-2008 at S2, S3 and S4. In 2007-2008 growing season, at S2 and S4, a significant further increase in HNT values was observed with the application of strobilurin fungicide at stem elongation (T3). In all the experiments the T5 treatment showed higher HNT values than the untreated control (T1). A significant effect of strobilurin fungicide application at heading on delaying senescence of the flag leaf was observed in 2006-2007 at S2, while no significant differences were observed in any experiments between the azole application at heading (T3) and the addition of a foliar N fertilizer (T4).

FHB symptoms and DON contamination

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

ANOVA showed a significant effect of foliar treatment on FHB incidence and severity in all the experiments, except for the FHB incidence in both growing seasons at S3. The azole application at heading (T2) reduced the FHB incidence and severity compared to the untreated control (T1), with a reduction of 14% and 11% for the two parameters, respectively.

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

The DON contamination was generally higher in grain samples harvested in 2008 than those of 2007. In all the experiments, a significant effect of the azole application was observed at heading (T2) on DON occurrence. The contamination of the T2 treatment was 51% lower than the T1 one. The addition of a foliar N fertilizer or the application of a strobilurin fungicide at the stem elongation stage did not show any significant differences for DON content at harvest compared to the azole programme at heading. On the other hand, the strobilurin fungicide application at heading led to a higher DON occurrence (36% higher) in grains harvested at S2 and at S4 in 2007-2008 compared to the T3 treatment. No significant differences were observed between this treatment (T5) and the untreated control (T1) in any of the experiments conducted in this growing season.

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

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

The test weight was generally lower in the grain samples harvested in 2008 than those of 2007, as a consequences of the higher FHB and foliar disease pressure. ANOVA showed significant differences between the treatments concerning the test weight in the experiments conducted at S2 in 2006-2007 and at S1, S3 and S4 in the 2007-2008 growing season. At S1 and S4 (2007-2008), the azole application at heading (T2) significantly increase, the test weight by 7% compared to the untreated control (T1). The test weight was significantly higher at S2 in 2006-2007 and at S1 and S3 in 2007-2008 when a strobilurin fungicide, applied at stem elongation (T3), was added to a azole programme at heading (T2). The application of a nitrogen foliar fertilizer or a strobilurin in addition at heading did not lead to any significant increase in test weight compared to T3 in any experiments.

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

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

Discussion

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

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

The azole application at heading led to a clearly higher grain yield, lower FHB symptoms and a lower DON contamination, in years as well high as low disease pressure. The increase in grain yield that was observed was mainly due to an incidence of shrivelled kernels. The results obtained for these fungicide treatments for FHB and DON control are in agreement with those obtained on common wheat by Koch *et al*. (2006) in Germany and by Blandino *et al*. (2006) in North Italy and on durum wheat by Menniti et al. (2003). Azoles have been confirmed to be effective against FHB infection and DON contamination, (Haidukowski *et al*., 2005) although in the second year of this research, with conditions of higher disease pressure, their application was not able to achieve a sufficient level of control of FHB. The cultivation of durum wheat in northern Italy therefore needs to take in account all the other agricultural practices that are able to minimize foliar disease damage and DON contamination risk in wheat grains. Precautionary measures to control STB and FHB infection should be taken into account at the beginning of wheat cultivation, especially in the regions more prone to fungal attacks, are avoiding cereal succession, incorporating the previous crop residues and using a resistant cultivar (Pirgozliev *et al*., 2003).

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

As expected, leaf greenness was influenced by fungicide, especially by the strobilurin application at stem elongation. These data confirms that delaying the senescence of flag leaves reduces the decline in physiological activity and could assure higher grain yields (Ruske et al., 2003a). Chlorophyll meters, such as HNT, have been confirmed to be useful instruments to describe the greenness status of flag leaves and they could predict differences in wheat yield, as reported by Vidal *et al*. (1999). The double strobilurin application (at the stem elongation stage and at heading) did not show any significant advantage on the control of the main foliar diseases, particularly STB, and, consequently, on grain yield, compared to the single application at the stem elongation stage. Moreover, according to Dimmock and Gooding (2002a), the addition of strobilurin to an azole programme at heading did not significantly delay senescence of the flag leaves, compared to the azole-only application. On the other hand, the application of this fungicide at heading led to an increase in DON contents that was often not significantly different from that of the untreated controls, confirming data collected in the same area in a previous trial on common wheat (Blandino *et al*., 2006). As suggested by Pirgozliev *et al*. (2003), the increase in DON observed with the strobilurin application is probably due to an increase in the infection of the *Fusarium* species, following the reduction of the presence of *Microdochium nivale*, a pathogen which, unlike other *Fusarium* species, is involved in the symptomatology of the disease, but which is not able to synthesise DON.

No effect of fungicide application on the protein content, ash concentration or vitreousness was recorded. The impact of STB control on the grain quality of common wheat has not been understood clearly either. Several authors (Peltonen and Karjalainen, 1992; Dimmock and Gooding, 2002b) have reported that the control of this foliar disease resulted in an increased wheat grain protein content, because of the prolonged duration of the green leaf area and the grain filling phase. Ruske *et al*. (2001) instead showed that protein concentrations were reduced significantly following *Septoria tritici* control. No significant effect of foliar fungicide application on grain protein was observed in other studies (Kelley et al., 1993; McKendry et al., 1995). Several studies have reported that if the crop is kept healthy, the photosynthetic period is prolonged and more of the nitrogen taken up by the plant can be translocated to the grain (Dimmok and Gooding, 2002a). On the other hand, conditions that promote leaf senescence during grain filling, such as foliar disease, tend to increase protein deposition over starch accumulation in the grain, because the production and translocation of carbohydrates to the grain is more sensitive to adverse growing conditions than protein production (Fernandez- Figares *et al*., 2000). Probably both these effects played a role in this study, thus no significant effect of disease control on grain protein content was observed.

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

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

In conclusion, the experiments conducted in 2 growing seasons have confirmed the criticality of the disease management of this crop in North Italy, especially in years with climatic conditions that are favourable to fungal pathogen development. Thus, the diffusion of this cereal to achieve high quality durum chains, in areas with weather conditions similar to those of the present study, could only be effective with a successful integrated programme to control foliar disease, FHB and DON contamination. At the moment, among the applied control methods, fungicide treatment plays an important and crucial but not decisive role. The double treatment, with a strobilurin application during the stem elongation stage and azole at heading, has emerged as an essential practice. Other foliar treatments at heading, such as strobilurin or foliar N fertilizer applications, did not seem to provide any further advantage, in either grain yield or quality. However, the application of strobilurin fungicide at heading could be a risky practice, since it could reduce the efficacy of azole fungicide to control DON contamination.

Although other studies are necessary to verify the effect of foliar treatment programmes on durum wheat in areas more prone to these fungal disease, and to check other and new fungicides, the cultivation of this crop in these regions could only be possible with the development and the selection of varieties which show a higher resistance to STB and FHB and the adoption of all the other preventive 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							
Site	S2		S3		S1		S2		S3		S4	
GS*	Rainfall (mm)	Rainy days (d)	Rainfall (mm)	Rainy days (d)	Rainfall (mm)	Rainy days (d)	Rainfall (mm)	Rainy days (d)	Rainfall (mm)	Rainy days (d)	Rainfall (mm)	Rainy days (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] T		STB severity[b] N (%)	HNT values (HNT unit) 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] T		FHB incidence[c] N (%)	FHB severity[d] T		FHB severity[d] N (%)	DON (μg kg^{-1})	
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√x*180/π) 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 μg kg^{-1}.

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

Table. 6

Effect of fungicide and foliar N applications on grain yield, test weight, grain protein content, ash content and vitreousness in the durum wheat kernels; field experiments 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
		P (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
		P (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
		P (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
		P (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
		P (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
		P (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
		P (F)	0.008	0.111	0.866	0.976	0.810

[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] Average data of 6 experiments conducted in 2