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FIELD CROP RESEARCH

Title: Enhancing grain yield and quality of winter barley through agronomic strategies to prolong canopy greenness.

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Abstract

An agronomic improvement in grain yield and quality in winter wheat could be obtained through the application of strategies, such as application of foliar fungicides or fertilizers, that protect health of the last leaves and delay the senescence process during ripening. Only a few studies have reported the effect of these practices on barley, although these treatments could represent a new opportunity to specialize in feed and food barley markets and raise farmer profitability.

The aim of this study was to compare the effect of different late-season strategies, N and S foliar fertilizers and fungicides applied at barley anthesis, on crop canopy greenness during the ripening stages and to establish the relationship between these strategies and barley yield and quality. Four field experiments have been conducted in NW Italy during 3 growing seasons, according to a full factorial design with 4 treatments, 3 barley cultivars and 4 replications. The following parameters were recorded: canopy greenness, grain yield, test weight (TW), thousand kernel weight (TKW), grain protein content (GPC), foliar disease incidence and severity and deoxynivalenol (DON) contamination. The collected data clearly underline the importance of prolonging canopy greenness of barley in order to increase grain yield and to improve quality. Of all the compared treatments, the application of a fungicide with an azole mixture at anthesis has shown to play the most important role in delaying the senescence process, and has resulted in a higher gran yield (+ 25%), TW (+ 1.3 kg hl^{-1}) and TKW (+ 2.8 g).

The effect of the fungicide treatment on barley grain yield was significant for all the compared cultivars and in almost all the environmental conditions, but led to a greater advantage in the cooler environments with prolonged ripening. The fungicide

also led to a clear, significant control of foliar disease and a reduction in DON contamination.

The use of N and S foliar fertilizers was able to prolong canopy greenness and enhance barley yield and quality but only in environments characterized by a prolonged grain filling period.

Keywords: winter barley, canopy greenness, fungicide, foliar fertilizer, deoxynivalenol.

Abbreviations: AUCGC, Area Under Canopy greenness Curve; DON, deoxynivalenol; FHB, Fusarium head blight; GDDs, growing degree days; GS, growth stage; GPC, grain protein content; STNB, Spot-type net blotch; TW, test weight; TKW, thousand kernel weight.

1. Introduction

Barley (*Hordeum vulgare* L.) is the fourth most important crop in the world, after wheat, maize and rice; in Italy the crop is equally distributed throughout the territory with 6.8 million t produced each year (Ansovini, 2009). Although this crop is characterized by a lower production cost than other cereals, and it can also be adapted to marginal environments, the barley surface has gradually been decreasing in the recent years. This reduction could be related to low trade prices, low incomes, poorly characterized supply chains and a lack of specific field programmes.

In Italy, winter hexastichous barley, which is normally used for animal feed is the most commonly grown type. However, the use of barley for food production, as pearled kernels, and for beverage in the malting industry, is increasing, and is generally based on distichous varieties. The use of barley in the food chain can be considered a new economic opportunity, but barley kernels with a high kernel size and test weight (TW) are required (Błażewicz et al., 2007) together with a low occurrence of contaminants (Lancova et al., 2008). Therefore, an improvement of the crop techniques in order to increase grain yield, grain protein content (GPC) and sanitation of this crop, could be a new opportunity to specialize feed and food barley and raise farmer profitability.

An improvement in yield and in the technological and sanitary quality in winter wheat could be obtained through the application of strategies that protect the health of the last leaves and delay the senescence process during ripening (Blandino and Reyneri, 2009).The duration of the photosynthetically active leaf area, for which the role of the last leaves is crucial, positively affects grain yield (Gooding et al., 2000; Zhang et al., 2010), the kernel size and its quality (Entz et al., 1990). The chlorophyll concentration

of the plant is related to several factors, but is primarily influenced by the N status in the leaves, and the N fertilization rate clearly affects canopy greenness.

The practice of adding a foliar N fertilizer at anthesis to a mineral fertilization programme could increase the duration of the wheat green leaf area, maintain canopy longevity during grain filling and enhance grain yield (Gooding et al., 2007). N applied as a foliar at anthesis has also shown to increase GPC in several experiments conducted with common and durum winter wheat (Gooding and Davies, 1992; Bly and Woodard, 2003).

A late-season S application, could also contribute by increasing canopy greenness and grain yield, as a positive interaction between N and S in wheat has been shown, which could reflect in a higher GPC (Pedersen et al., 1998; Luo et al. 2000).

As far as biotic stress is concerned, the occurrence of fungal foliar diseases could clearly negatively influence greenness canopy duration. A reduction in foliar disease development leads to yield benefits, related to a longer period of green leaf area and higher absorption of photosynthetically active radiation by healthy green tissues (Waggoner and Berger, 1987). The disease spot-type net blotch (STNB) of barley caused by *Drechslera teres* f. *maculata* (Sacc.) has become one of the most important worldwide foliar diseases of this crop (Jayasena et al., 2002). Spores are produced on infected plants and are spread by wind or rain, and cooler temperatures and higher moisture levels favour infection (Tekauz, 1986). STNB causes a net-type lesion, which is characterized by dark brown blotches with a net-like pattern and the presence of chlorosis (Richter et al., 1998).

Fungicides could be used to control this disease, although they are generally rarely applied on barley by farmers. Among the various fungicides that are available, azole applications at anthesis have shown to have a significant effect on the decline of the

green leaf area in wheat (Kettlewell et al., 1982) and on the increase in grain yield and kernel weight (Egli, 1998). However, some azoles have shown beneficial effects that are not only attributable to disease control, but also related to a physiological action on plants (Riesen and Close, 1987). The use of fungicides at anthesis also plays a key role in obtaining wheat grains with a low deoxynivalenol (DON) contamination, especially in environments in which there is a high frequency of Fusarium head blight (FHB) (Blandino et al., 2012). This disease can be produced by several species of *Fusarium* but *F. graminearum* (Schwabe) is the principal barley infecting species. The maximum permissible levels of DON contamination in barley have been established as 1250 μg kg^{-1} for food (EC, 2006b) and at 8000 μg kg^{-1} for feeds (EC, 2006a).

Since winter barley is characterized by a higher precocity and a shorter ripening time in temperate areas, compared to winter wheat, the aim of this study was to verify whether the application of agricultural strategies that are able to prolong crop greenness could also lead to advantages for this cereal. Another aim of this study was to compare the effect of different late-season strategies, N and S foliar fertilizers and fungicide applications at barley anthesis, on crop canopy greenness during the ripening stages and to establish the relationship between these strategies and barley yield and quality.

2. Materials and methods

The study has been carried out in North West Italy (Piedmont region), during 3 growing seasons (2010-2011, 20011-2012, 2012-2013) at Cigliano, (VC, 45° 14' N, 8° 00' E; at an altitude of 194 m, in a shallow and sandy soil -Typic Hapludalfs) and during one growing season (2012-2013) at Cuneo (44° 23' N, 7° 32' E; at an altitude of 480 m; in a deep and fertile sandy soil - Typic Eutrochrepts).

The main crop and agronomic information relating to the experimental fields is reported in Table 1, while the main soil properties are reported in Table 2.

Different foliar treatments were applied at mid anthesis [growth stage (GS) 65] (Zadoks et al., 1974) in order to reduce the canopy senescence process and prolong the grain filling period:

- a nitrogen (N) foliar fertilizer (T3);
- a sulfur (S) and nitrogen (N) foliar fertilizer (T4);
- a fungicide mixture, with physiological activity (T5)

The treatments were compared with an untreated control at the same N fertilizer rate (T2). Moreover, another untreated control, which received half of the N fertilizer rate of the other treatments (T1), was introduced as a spy-control, in order to obtain a term of comparison for the foliar application effect on greenness and for the productive and qualitative parameters in the different environmental conditions. The following active ingredients or products were used:

- Foliar N fertilizer: YaraVita ™ Last® N (Yara S.p.A., Milano, Italy), 312 g N l^{-1} (25%), applied at 5 kg N ha^{-1} (15 l of product formulation ha^{-1});

- Foliar N and S fertilizer: Sulfamon® (Cifo S.p.A., S. Giorgio di Piano, BO, Italy), 100 g N l^{-1} (8%) and 1275 g S l^{-1} (22%), applied at 0.4 kg N ha^{-1} and 5 kg S ha^{-1} (4 l of product formulation ha^{-1});
- fungicide: prothioconazole + tebuconazole (Prosaro®, Bayer CropScience S.r.l., Milan, Italy, formulation: emulsifiable concentrate) both applied at 0.125 kg active ingredient (AI) ha^{-1};

The complete treatment schedule is summarized in Table 3. The application of fungicides or a foliar fertilizer to winter barley is not a common crop technique in Italy. The treatments were compared in each trial containing 3 winter barley varieties: cv. Ketos (Limagrain Italia Spa, Busseto, PR, Italy), a hexastichous, late maturity variety, intended mainly for feeds, cv. Cometa (Apsovsementi, Voghera, PV, Italy), a distichous medium maturity variety, intended mainly for feeds, and cv. Sfera (Centro di ricerca per la genomica e la postgenomica animale e vegetale, Fiorenzuola d'Arda, PC, Italy), a distichous early maturity variety intended mainly for malting and food.

The experimental design was a randomized complete block with four replicates. The plot size was 7 m x 1.5 m.

The previous crop in each trial was maize for grain, which is the most common cropping system in the area. The seed bed was set after ploughing (30 cm) and harrowing. Planting was conducted in 12 cm wide rows, at a seeding rate of 400 seeds m^{-2}. Weed control was achieved with isoproturon and diflufenican applied at barley tillering (GS 31). The foliar fertilizers and fungicides were applied with a three nozzle precision sprayer (T-Jet 110/04), using a fine mist at a slow walk to ensure effective coverage. The delivery pressure at the nozzle was 324 KPa. With the exception of the T1 treatment, a total of 130 kg N ha^{-1} was applied to the plots as granular ammonium nitrate fertilizer, split between 50 kg N ha^{-1} at GS 25 and 80 kg N

ha^{-1} at GS 33. The N rate for the T1 treatment at each application time was 25 and 40 kg N ha^{-1}, respectively. The sowing, foliar application and harvesting date for each trial are reported in Table 1.

The grain yields were determined at crop maturity, using a Walter Wintersteiger cereal plot combine-harvester. A subsample was taken from each plot to determine the grain moisture, test weight (TW) and thousand kernel weight (TKW). The grain yield results were adjusted to a 120 g kg^{-1} moisture content.

The harvested grain~~s~~ was thoroughly mixed, and 2 kg grain samples were taken from each plot to analyze the grain protein content (GPC).

2.1 Canopy greenness

A hand-held optical sensing device, GreenSeekerTM® (Trimble©, Sunnyvale, California, USA), was used to measure the relative photosynthetically active biomass from heading to the end of grain filling stage, after the foliar treatment application. This method is non destructive and can be conducted in real time. The GreenSeekerTM® device uses to monitor active radiation to obtain reflectance data that is independent of the solar illumination. It has its own consistent light emission source, photodiode detectors and interference filters for red [R] and near infrared [NIR] wavelengths at the 671±6 nm and 780±6 nm spectral bands, respectively (Jordan, 1969); it provides the Normalized Difference Vegetation Index (NDVI) values, which are calculated as follows (Rouse et al., 1974):

$$NDVI = \frac{R_{NIR} - R_{Red}}{R_{NIR} + R_{Red}}$$

where R_{NIR} is the NIR radiation reflectance and R_{Red} is the visible red radiation reflectance.

The NDVI measurements were carried out every 7 days from the end of heading (GS 58) to senescence (GS 90). The instrument was held approximately 80 cm above the canopy and its effective spatial resolution was 2 m^2. The NDVI values are proportional to the crop biomass and the greenness. Since, in the present experiment, the measurements were conducted starting from barley heading, when the crop biomass reaches a maximum and remains almost stable until harvest, the NDVI values are proportional to the amount of total chlorophyll in the crop canopy and can be considered descriptors of the crop senescence process.

The Area Under Canopy Greenness Curve (AUCGC) was calculated for each treatment starting from the NDVI measurement for all the observation dates and using the following formula:

$$AUCGC = \sum_{i}^{n-1} \{[(R_i + R_{i+1})/2]\,(t_{i+1} - t_i)\}$$

where R is the NDVI reading value, t is the time of observation and n is the number of observations.

2.2 Foliar disease symptoms

All the experiments relied on natural infection. STNB incidence and severity were recorded in the T2 and T5 treatments, on the basis of a evaluation of the disease at the soft dough stage in each plot (GS 85). STNB incidence was calculated as the percentage of leaves with symptoms that were recorded when the two last emerged leaves of 15 randomly selected plants were analyzed. STNB severity was computed as the percentage of leaf surface with symptoms, using a standard area diagram

(James, 1971; modified). A scale of 1 to 7 was used to show the percentage of surfaces exhibiting visible symptoms of the disease, where: 1 = no symptoms, 2 = 0-2%, 3 = 2-5 %, 4 = 5-10%; 5 = 10-25 %, 6 = 25-50%, 7 = 50-100%. The scores were converted to percentages of the leaf exhibiting symptoms, and convert to the mid-point of the interval.

2.3 Quality parameters

The moisture concentration and the TW were determined by means of a Dickey-John GAC2100 grain analysis meter (Dickey-John Corp. Auburn, IL, USA) using the supplied programme. Calibration for moisture was checked using oven drying techniques. Two hundred kernels were randomly collected from each 1kg sample and weighed using an electronic scales to assess the TKW. Grain protein content (GPC) was determined by near-infrared transmittance technology, using a Infratec™ 1241 Grain Analyser instrument (Foss, Silver Spring, MD, USA) and presented on a dry matter basis.

A 2 kg representative sample of grain from the plots of the T2 and T5 treatments was freeze-dried and milled. A 20 g representative sub-sample of the milled material was analyzed for DON. The DON contaminations were analyzed according to the ELISA method, Ridascreen® DON (R-Biopharm AG, Darmstadt, Germany). Briefly, DON were extracted from 20 g samples in a glass centrifuge bottle with 100 ml of distilled water by shaking for 15 min. The supernatant was filtered through Whatman® No. 1 filter paper and 50 µl of the filtrate (range 18.5 µg kg^{-1} – 500 µg kg^{-1}) or a 1:10 diluted filtrate (range 185 µg kg^{-1} - 5000 µg kg^{-1}) were used for photometrical quantification at 450 nm, using a plate reader (Digital & Analog Systems srl, Ralombara Sabina, RM; Italy).

2.4 Statistical analysis

The normal distribution and homogeneity of variances were verified for each trial by performing the Kolmogorov–Smirnov normality test and the Levene test, respectively. An analysis of variance (ANOVA) was utilized to separately compare the AUCGC values, grain yield, TW, TKW and GPC, for each trial, using a ~~completely randomized block~~ randomized complete block design, in which the treatment and the variety were the independent variables. Multiple comparison tests were performed according to the REGW-Q test on treatment means. ANOVA was utilized to compare the effect of fungicide application to STNB incidence and severity, and DON content, considering only the T2 and T5 treatments for each trial. The STBN incidence and severity values were arcsin square root transformed before any further statistical analysis, as percentage data derived from counting. The DON concentration was transformed using the $y'=\ln(x+1)$ equation to normalize the residuals. The SPSS statistical package for Windows, Version 20.0 (SPSS Inc., Chicago), was used for the statistical analysis.

3. Results

The compared field trials showed different meteorological trends, mainly during the ripening stages (Table 4).

There was very little rainfall as well as high temperature (expressed as growing degree days, GDD) at Cigliano in 2010-2011 growing season, from the stem elongation to anthesis stage, while frequent rainfall occurred at the end of ripening, after the soft dough stage, although grain filling duration was not prolonged.

The precipitation was instead frequent and regular from April to June at Cigliano in 2011-2012 growing season, but there was a high number of GDD from GS 45 to GS 87 and this led to quick crop maturation.

Frequent rainfall and low temperatures occurred at Cigliano and Cuneo in 2012-2013 growing season during the late vegetative and the grain filling stages. The coolest conditions during the grain filling stage were recorded at Cuneo in 2012-2013 growing season, and led to the harvest being delayed till the middle of July.

3.1 Canopy greenness

The NDVI readings clearly describe the greenness status of the canopy in the different treatments and trials (Figure 1). Generally, the NDVI values decreased with progressive crop maturation and senescence. Canopy greenness (NDVI values) showed a similar trend for all the treatments up to the end of heading (GS 60), while differences were observed between the different treatments from anthesis onwards. As expected, the spy-control with the half N rate applied thorough soil fertilization (T1) showed the lowest values and led to an early crop senescence. Differences in the NDVI values between the different foliar application strategies were particularly

evident from the development of seeds (GS 70) to medium dough (GS 85): during these stages, the fungicide application (T5) showed the highest greenness canopy in all the trials. The dry growing season at Cigliano in 2010-2011 growing season led to the lowest NDVI values and the most rapid curve slope decrease. On the other hand, at Cigliano and ~~D~~ Cuneo in 2012-2013 growing season, the wetter and cooler climatic conditions led to higher NDVI values and slower ripening duration. Cigliano in 2011-2012 growing season showed an intermediate trend.

The mean AUCGC values for each treatment and variety are reported separately for each trial in Table 5. ANOVA showed significant differences ($P<0.001$) between treatments, for the AUCGC values in all the compared trials. No significant differences were observed between treatments T2, T3, T4 and T5 at Cigliano in 2010-2011 growing season, where only the low fertilized control (T1) resulted in a significantly lower AUCGC value. On the other hand, the fungicide application at anthesis (T5) significantly increased AUCGC compared to the untreated control (T2) at Cigliano in 2011-2012 and 2012-2013 growing seasons and at Cuneo in 2012-2013 growing season. The capacity of the fungicide application to increase canopy greenness was higher in the trials in which the climatic conditions were able to prolong the grain filling period. In fact, the fungicide application increased the AUCGC by 6%, 4% and 13% for trials carried out at Cigliano in 2011-2012 and 2012-2013 growing seasons and at Cuneo in 2012-2013 growing season, respectively.

The application of N foliar fertilizer (T3) at Cigliano in 2011-2012 growing season significantly increased the AUCGC compared to the untreated control (T2), while at Cuneo in 2012-2013 growing season both foliar fertilizer applications (T3 and T4)

significantly prolonged canopy greenness compared to treatment T2, although the effect of these treatments lasted for a shorter time than the fungicide application.

cv. Sfera, which has high precocity, showed significantly lower AUCGC values than cv. Ketos, which, since it is a late maturity variety, resulted in a delayed senescence process. cv. Cometa confirmed an intermediate precocity.

No significant interaction effects between treatments and variety were observed in any of the trials.

3.2 Grain yield and kernel quality parameters

The grain yield for each treatment and variety are reported, separately for each trial, in Table 5. ANOVA showed a significant difference ($P<0.001$) between treatments in all the compared trials. No significant differences were observed between treatment T2, T3, T4 and T5, at Cigliano in 2010-2011 growing season, while only the spy-control (T1) resulted in a significant yield decrease of 20%, compared to previous treatments.

The fungicide application (T5) at Cigliano in 2011-2012 and 2012-2013 growing seasons and at Cuneo in 2012-2013 growing season, significantly increased the grain yield, compared to the untreated control (T2), by 20%, 9% and 49%, respectively. The foliar fertilizer application at anthesis did not increase grain yield in experiments carried at Cigliano in 2011-2012 and 2012-2013 growing seasons, while the application of both foliar fertilizer (T3 and T4) significantly increased grain yield at Cuneo in 2012-2013 growing season, compared to the untreated control (T2). However, the fungicide application also led to a significantly higher yield compared to both foliar fertilizers in this trial. No significant difference was observed between the compared cultivars at Cigliano 2010-2011 and 2011-2012 growing seasons. On the

other hand, in trial carried out at Cigliano and Cuneo in 2012-2013 growing season, which were characterized by higher rainfall during the late vegetative and grain filling stages, the late maturity cv. Ketos, showed significantly higher grain yield, compared to the other varieties. No significant effects of the interaction between treatments and variety were observed in any of the trials.

With the exception of experiment carried out at Cigliano in 2011-2012 growing season, a significant regression between AUCGC and grain yield values was observed in each experiment (Cigliano, 2010-2011: R^2 =0.863, *P*=0.022; Cigliano 2012-2013: R^2 =0.971, *P*=0.002; Cuneo 2012-2013: R^2=0.928, *P*=0.008).

The mean TW and TKW values for each treatment and variety are reported, separately for each trial, in Table 5. With the exception of TKW at Cigliano in 2010-2011 growing season, ANOVA showed a significant difference between treatments in all the compared trials for both parameters. The application of a double mineral N rate (T2), at Cigliano in 2011-2012 growing season and Cuneo in 2012-2013 growing season, compared to the spy-control (T1), resulted in a significantly average TW increase of 1.2 kg hl^{-1}. The fungicide application (T5) significantly increased the TW by 2.1 and 1.3 kg hl^{-1}, compared to the untreated control (T2), at Cigliano in 2011-2012 growing season and Cuneo in 2012-2013 growing season, respectively. No differences in TW were observed for either foliar fertilizer (T3 and T4), compared to the untreated control (T2).

The distichous cv. Sfera showed higher TW values than the other cvs. in all the trials. Only at Cigliano in 2010-2011 and 2011-2012 growing season, did the hexasticous cv. Ketos showed show a significantly lower TW than cv Cometa.

A significant effect of the interaction between the treatments and variety was only observed at Cigliano in 2011-2012 growing season. cv. Ketos did not show any

differences between treatments, while the fungicide application significantly increased the TW for cvs. Cometa and Sfera (data not shown).

The fungicide application (T5) significantly increased the TKW values compared to the untreated control (T2) at Cigliano in 2011-2012 and 2012-2013 growing seasons and at Cuneo in 2012-2013 growing season. The average increase was by 4.4, 2.6 and 3.2 g in trials–carried out at Cigliano in 2011-2012 and 2012-2013 growing seasons and at Cuneo in 2012-2013 growing season, respectively. The distichous cv. Cometa reported the highest TKW in all the trials. ANOVA showed a significant difference ($P<0.001$) between treatments at Cigliano in 2010-2011 and 2012-2013 growing seasons and at Cuneo in 2012-2013 growing season for GPC (Table 5).

The untreated control (T2) resulted in a significant increase in GPC in all these experiments, compared to the spy-control with the half N fertilization rate (T1), while no significant difference was observed between either foliar fertilizer (T3 and T4) and T2. Only at Cuneo in 2012-2013 growing season, did the fungicide application (T5) significantly affect the GPC: the protein content was significantly lower in the fungicide treated plots, compared to the untreated control (T2). cv. Cometa generally showed a higher GPC than cv. Ketos.

No significant differences were observed for GPC for the interaction between treatment and variety in any experiment.

3.3 STNB symptoms and DON contamination

The incidence and severity of the STNB symptoms recorded during the visual evaluations of the last two emerged leaves at the soft dough stage are report in Table 6. No foliar symptoms of other fungal diseases, such as rhizoctonia or rust were observed.

With the exception of the STNB incidence at Cuneo in 2012-2013 growing season, ANOVA showed a significant effect of the fungicide treatment on both disease parameters. The application of a mixture of prothioconazole + tebuconazole at flowering (T5) significantly reduced STNB incidence, compared to the untreated control (T2); the decrease in incidence percentage was 40, 48 and 36%, respectively for trials–carried out at Cigliano in 2010-2011, 2011-2012 and 2012-2013 growing seasons. The T5 treatment had a significantly lower STNB severity than the untreated T2 in all trials. The capacity of the fungicide to control the disease was higher at Cigliano in 2010-2011, 2011-2012 and 2012-2013 growing seasons, with a 95% higher average reduction of disease severity than experiment D, in which the reduction in the disease symptoms was 74%.

The compared cvs. did not show a significant different disease susceptibility at Cigliano in 2010-2011 and 2012-2013 growing seasons and at Cuneo in 2012-2013 growing season. The late maturity cv. Ketos showed a higher disease incidence and severity at Cigliano in 2011-2012 growing season, than the other cvs.

The occurrence of DON in the barley grain samples was clearly affected by the climatic condition of the growing season: at Cigliano in 2011-2012 growing season, the average occurrence of this mycotoxin was above 1250 ug kg^{-1} (Reg. CE 1881/2006), while it was lower than 500 ug kg^{-1} in the other trials. With the exception of trial ~~A~~ carried out at Cigliano in 2010-2011 growing season, which was characterized by drier climatic conditions from anthesis to the end of the ripening stage, ANOVA showed a significant reduction in DON contamination in the grain for the fungicide application compared to the untreated control (T2). The application of the prothioconazole + tebuconazole mixture, applied at anthesis, reduced the DON contamination by 79%, 60% and 59%, at Cigliano in 2011-2012 and 2012-2013

growing seasons and at Cuneo in 2012-2013 growing season respectively. Significant differences between cv. were observed for DON contamination in all the trials, with the exception of trial B: the late maturity cv. Ketos resulted in the highest DON occurrence.

No significant differences were observed for the interaction between treatment and variety in any experiment.

4. Discussion

The collected data suggest that, in temperate areas, the yield and quality of winter barley could be related to the duration of canopy greenness during the grain filling period. In this experiment, the delay of leaf senescence clearly prolonged the crop ripening stages and positively influenced the grain yield, the TW and the TKW.

The pedo-climatic conditions resulted in the greatest effect on canopy greenness duration: as also reported by Aparicio et al., (2000), this parameter is positively influenced by cooler climatic conditions and rainfall during grain filling.

Furthermore, the present manuscript has clearly underlined that agronomical practices, such as foliar applications of fertilizers and fungicides at crop anthesis, could also play an important role in prolonging canopy greenness, but their influence is influenced to a greater extent by the environmental conditions during the ripening stages.

Of all the compared treatments, the fungicide application has led to the best results, in terms of a prolongation of barley canopy greenness. This result is in agreement with previous reports that azoles produce a longer green leaf duration of wheat leaves than an untreated control (Gooding et al., 2000; Ruske et al., 2003). Spot-type net blotch has been confirmed to play an important role in reducing canopy greenness in barley cultivated in temperate areas. Furthermore, the application of a fungicide at flowering causes a clear delay in barley canopy senescence, which is not only due to the direct control of foliar disease, but that could also be related to a probable physiological effect. Certain fungicides classes could prolong canopy greenness through physiological effects, such as promoting the growth hormone cytokinin and delaying the inhibitor ethylene (Grossmann and Retzlaff, 1997). As far

as the physiological effect of prothioconazole is concerned, in an experiment conducted under disease free-conditions in a greenhouse, Berdugo et al. (2012) reported beneficial effects on leaf physiology in wheat plants. The senescence process, due to disease foliar development, is closely related to the breakdown of the chlorophyll and chloroplasts, which result in a decline in photosynthetic activity (Lingrui et al., 2007). Moreover, the delay in leave senescence in cereals could also be attributed to the control of saprophytic fungi on the plant surface through a fungicide application (Bertelsen et al., 2001). Tolstrup (1984) reported that saprophytic fungi may also influence leaf senescence in barley.

The delay in greenness obtained through the fungicide application led to a more uniform ripening and a longer grain fill period, which led to higher grain yield, TW and TKW values. The effect of the fungicide treatment on barley grain yield was significant for all of the compared cultivars and in almost all the environmental conditions. Only at Cigliano in 2010-2011 growing season, in the presence of environmental conditions that led to a rapid ending of crop ripening, did the fungicide application not lead to a significant increase in the grain yield and yield parameters. Moreover, the effect of fungicide on enhancing grain yield can clearly be seen moving from the short grain filling period to the prolonged one.

A direct relationship between the extension of greenness canopy through fungicide application and higher winter wheat grain yield has been shown in several studies (Gooding et al., 2000; Jörgensen and Olensen, 2002). Spilde (1989) reported that the fungicides that were most effective in controlling foliar disease in cereals also clearly increased the seed size. This effect is particularly interesting as far as the malting industry is concerned, since this transformation of barley requires large kernels,

associate with a low GPC, in order to obtain a high malt yield (Dragovi and Maleˇ, 1998).

The use of feed barley with a high GPC could be an opportunity for breeders to reduce the costs of livestock rations, as this could lead to a reduction in the use of more expensive additive ingredients and other protein supplements. In this experiment, the fungicide application did not affect the GPC of the barley, with the exception of experiment carried at Cuneo in 2012-2013 growing season, where the important increase in grain yield led to a dilution of the protein. Wheat fungicide applications at anthesis could also lead to a dilution of protein, as a consequence of a higher grain yield in the environmental conditions that allow a prolonged ripening stage. (Ruske et al., 2003). On the other hand, the observed delay in canopy senescence did not increase the GPC of the compared barley cultivars, even in the environment with the largest ripening period. The application of a fungicide mixture to wheat, that is able to provide a physiological benefit for the crop showed a significant increase in GPC (Blandino and Reyneri, 2009). Since barley is characterized by a higher precocity and a lower capacity to accumulate protein in the kernels, the effect of a canopy senescence delay on the protein content in grain is expected to be less obvious than in wheat.

In addition to the yield increase, the fungicide application clearly improved the sanitary parameters through a reduction in mycotoxin contamination. Although DON contamination for wheat is a known risk (Jones, 2000), it has not been fully recognized in barley, except in brewing industries (Pan et al., 2007). The barley grains harvested in the different experiments always showed a lower DON content than the admissible maximum levels for feeds (8000 μg kg^{-1}; EC, 576/2006), but in

some environmental conditions (e.g. Cigliano at 2011-2012 growing season), the contamination was higher than the food threshold (1250 μg kg^{-1} ; EC, 1881/2006). Furthermore, the application of prothioconazole + tebuconazole at anthesis significantly reduced the DON content in all the experiments, even in the years with a much lower disease pressure and average contamination of this mycotoxin. These data clearly underline that the cultivation of barley for malting and other food chains in the typical climatic conditions of North Italy requires a careful control of the content of this mycotoxin. The collected data have shown that the fungicide application at anthesis is a crop practice that could be taken into consideration in environments and growing seasons with climatic conditions that could favour DON occurrence. Of the azole group, prothioconazole has been reported to be the most effective fungicide in controlling *Fusarium* spp. and in reducing the DON level in wheat grain (Paul et al., 2008).

As far as the efficacy in prolonging the canopy greenness of barley is concerned, the application of N or N-S as a foliar fertilizer at flowering seems to exert a less important effect in increasing AUCGC during grain filling than a fungicide. The application of foliar fertilizers, only leads to significant benefits in grain yield and quality in environments characterized by a very wet climatic condition during ripening and longer grain filling period. Also in these conditions, these foliar application of fertilizer lead to a quicker canopy senescence than the fungicide application.

The use of an N or NS foliar fertilizer at anthesis, at the rate applied in the present experiment, did not increase the GPC in barley. The use of a late-applied foliar fertilizer on common and durum wheat showed conflicting results concerning the protein content which are closely related to the rate and pedo-climatic conditions

(Blandino and Reyneri, 2009; Blandino et al. 2009; Garrido-Lestache et al., 2005; Tea et al., 2007).

In conclusion, as established for wheat in recent years, it is also necessary to reconsider the crop system for barley and to look for field programmes that are able to increase the productivity and quality of this cereal in an efficient and sustainable way for the different growing areas. For temperate areas, such as North Italy, the data collected clearly underline the importance of prolonging the canopy greenness of barley in order to increase the grain yield and improve quality. Among the treatments evaluated, it has been shown that a fungicide application at anthesis could play an important role and lead to clear advantages in terms of grain yield, quality (high TW and TKW) and low mycotoxin contamination. It has also been shown that the use of foliar fertilizers, containing N and S, leads to prolonged canopy greenness and enhanced barley yield and quality, but only in environments characterized by a prolonged grain filling period.

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Table 1.

Treatments compared in the 4 experimental trials conducted in the 2010-2013 period in North West Italy.

Treatment	N fertilization[a] (kg N ha^{-1})		Foliar application
	tillering GS[b] 25	stem elongation GS 33	flowering GS 62
T1	25[c]	40	-
T2	50	80	-
T3	50	80	N fertilizer[c]
T4	50	80	N + S fertilizer[d]
T5	50	80	Fungicide[e]

[a] Using granular ammonium nitrate (27%) fertilizer.

[b] Growth stage (Zadocks et al., 1974).

[c] The applied foliar N fertilizer was YaraVita TM Last® N (Yara S.p.A., Milano, Italy), composition: 312 g N l^{-1} (25%); application rate: 11 l ha^{-1}.

[d] The applied foliar N and S fertilizer was Sulfamon® (Cifo S.p.A., S. Giorgio di Piano, BO, Italy), composition 100 g N l^{-1} (8%) and -1275 g S l^{-1} (22%); application rate: 4 l ha^{-1}.

[e] The applied fungicide was a mixture of prothiconazole and tebuconazole (Prosaro®, Bayer Crop Science S.r.l., Milan, Italy, formulation: emulsifiable concentrate), both applied at 0.125 kg of active ingredient (AI) ha^{-1}.

Table 2.

Main agronomical and phenological information of the 4 experimental trials conducted in the 2010-2013 period in North West Italy.

Parameter	Site	Growing season	Growing Stage[a]								
			Sowing	Tillering	Stem elongation	Heading	Flowering	Medium milk	Medium dough	Senescence	Harvest
			-	25	33	58	65	75	85	90	-
Date	Cigliano	2010-2011	5/11/2010	8/03/2011	5/04/2011	8/05/2011	17/05/2011	31/05/2011	8/06/2011	20/06/2011	27/06/2011
	Cigliano	2011-2012	5/11/2011	1/03/2012	2/04/2012	3/05/2012	11/05/2012	24/05/2012	8/06/2012	14/06/2012	22/06/2012
	Cigliano	2012-2013	6/11/2012	11/03/2013	15/04/2013	10/05/2013	21/05/2013	5/06/2013	17/06/2013	25/06/2013	2/07/2013
	Cuneo	2012-2013	3/11/2012	21/03/2013	3/05/2013	23/05/2013	29/05/2013	14/06/2013	3/07/2013	9/07/2013	15/07/2013
GDD[b]	Cigliano	2010-2011	0	576	880	1403	1567	1848	1955	2237	2417
	Cigliano	2011-2012	0	607	995	1369	1505	1714	2023	2142	2327
	Cigliano	2012-2013	0	658	956	1385	1567	1818	2099	2303	2454
	Cuneo	2012-2013	0	749	1179	1460	1533	1807	2196	2332	2490

[a] Growth stage (Zadocks et al., 1974).

[b] Accumulated growing degree day using a 0°C base.

Table 3.
Main physical and chemical characteristics of the soil[a] in the 4 field experiments conducted in the 2010-2013 period in North West Italy.

Parameters / Site / Growing season	Cigliano 2010-11	Cigliano 2011-12	Cigliano 2012-13	Cuneo 2012-13	Measure unit
Sand (2 -0.05 mm)	36.6	36.3	43.1	39.9	%
Silt (0.05 - 0.002 mm)	52.6	52.5	45.6	49.6	%
Clay (< 0.002 mm)	10.8	11.3	11.5	10.5	%
pH	6.2	6.7	6.1	7.5	
Organic matter	1.76	1.69	1.54	2.35	%
C/N	10.0	10.2	10.6	8.0	
Cation Exchange Capacity (C.E.C.)	10.8	9.55	8.85	10.85	meq/100g
Total Nitrogen	0.103	0.097	0.085	0.123	
Exchangeable Potassium	38	44	92	242	ppm
Available Phosphorus (P Olsen)	15	16	78	34	ppm

[a] Soil was sampled from 0-30 cm

Table 4.

Decade rainfall and growing degree days (GDD[a]) from April to July 2010-2013 for the 4 experiments.

Month	Decade	Cigliano 2010-2011			Cigliano 2011-2012			Cigliano 2012-2013			Cuneo 2012-2013		
		Rainfall (mm)	Rainfall days	GDD (°C d^{-1})	Rainfall (mm)	Rainfall days	GDD (°C d^{-1})	Rainfall (mm)	Rainfall days	GDD (°C d^{-1})	Rainfall (mm)	Rainfall days	GDD (°C d^{-1})
April	I	0	0	181	59	7	121	44	5	89	29	7	68
	II	0	0	141	71	9	100	37	4	158	13	3	142
	III	59	7	153	24	4	139	83	7	151	83	9	129
May	I	0	1	169	37	4	165	24	8	174	36	8	157
	II	6	3	185	34	2	161	121	8	150	88	6	128
	III	23	3	224	56	4	215	16	8	156	11	4	138
June	I	136	10	183	32	5	205	10	3	193	12	3	176
	II	57	6	209	31	4	217	3	2	237	3	3	219
	III	4	4	235	7	2	248	3	3	195	3	2	190
July	I	-	-	-	-	-	-	-	-	-	7	6	222
GS 45 - 90		287	34	1679	352	41	1571	298	43	1413	243	41	1360

[a] Accumulated growing degree days for each month using a 0°C base .

Table 5.

Effect of N fertilization and fungicide protection on the barley area under canopy greenness curve (AUNTC), grain yield, test weight (TW), thousand kernel weight (TKW) and grain protein content (GPC); field experiments conducted in North West Italy in the 2010-2013 period.

1

Experiment	Factor	Source of variation	AUCGC	Grain Yield (t ha^{-1})	TW (kg hl^{-1})	TKW (g)	GPC (%)
Cigliano 2010-2011	Treatment[a]	T1	23.5 b	4.1 b	64.8 b	28.7 a	10.7 b
		T2	27.1 a	5.0 a	66.1 ab	26.9 a	11.8 a
		T3	27.9 a	4.9 a	67.1 a	28.0 a	11.9 a
		T4	26.8 a	4.9 a	65.8 ab	27.8 a	12.0 a
		T5	27.9 a	4.9 a	66.7 a	27.6 a	11.9 a
		P (F)	**<0.001**	**<0.001**	**0.009**	0.475	**<0.001**
		sem[b]	2.5	0.9	2.5	4.2	0.9
	Variety	Ketos	28.0 a	4.6 a	62.5 c	26.0 c	11.5 b
		Cometa	25.7 b	4.6 a	66.8 b	29.7 a	11.9 a
		Sfera	26.1 b	4.9 a	68.9 a	27.7 b	11.7 ab
		P (F)	**<0.001**	0.137	**<0.001**	**<0.001**	**0.048**
		sem[b]	1.9	0.7	1.9	9.2	0.7
		Treat x var	0.404	0.869	0.939	0.523	0.987
		sem[b]	4.3	1.6	4.3	7.2	1.5
Cigliano 2011-2012	Treatment	T1	22.2 d	5.8 d	66.4 c	45.9 b	11.9 a
		T2	25.1 c	6.7 b	67.9 b	43.3 c	11.8 a
		T3	25.7 b	6.4 bc	68.1 b	44.5 bc	12.2 a
		T4	24.9 c	6.2 c	67.7 b	43.7 c	11.5 a
		T5	26.6 a	7.8 a	70.0 a	47.8 a	11.6 a
		P (F)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.105
		sem[b]	1.1	0.6	1.9	2.9	0.9
	Variety	Ketos	24.7 b	6.5 a	65.8 c	40.5 c	12.0 a
		Cometa	25.9 a	6.5 a	68.1 b	49.6 a	11.6 a
		Sfera	24.0 c	6.7 a	70.1 a	45.5 b	11.8 a
		P (F)	**<0.001**	0.176	**<0.001**	**<0.001**	0.287
		sem[b]	0.8	0.5	1.5	2.3	0.7
		Treat x var	0.837	0.279	**<0.001**	0.974	0.574
		sem[b]	1.9	1.2	3.3	5.2	1.7
Cigliano 2012-2013	Treatment	T1	27.1 c	5.9 c	68.3 a	49.1 c	9.5 b
		T2	31.6 b	7.4 b	69.7 a	49.9 bc	10.5 a
		T3	31.1 b	7.4 b	69.9 a	50.6 b	10.8 a
		T4	31.3 b	7.6 b	69.4 a	50.2 bc	10.5 a
		T5	32.6 a	8.1 a	70.0 a	52.5 a	10.6 a
		P (F)	**<0.001**	**<0.001**	0.068	**<0.001**	**<0.001**
		sem[b]	2.2	0.7	2.2	2.3	0.6
	Variety	Ketos	31.2 a	7.7 a	68.2 b	50.2 b	10.1 b
		Cometa	31.9 a	7.0 b	68.6 b	53.5 a	10.8 a
		Sfera	29.3 b	7.2 b	71.5 a	47.9 c	10.0 b
		P (F)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
		sem[b]	1.7	0.5	1.7	1.8	0.4

		Treat x var	0.848	0.611	0.168	0.641	0.937
		sem[b]	3.8	1.2	3.9	4.0	1.0
Cuneo 2012-2013	Treatment	T1	18.8 d	7.0 c	67.4 c	51.2 b	11.0 c
		T2	22.2 c	7.5 c	68.1 bc	51.4 b	12.1 a
		T3	24.6 b	9.9 b	68.9 ab	53.1 ab	12.1 a
		T4	25.4 b	10.0 b	68.9 ab	53.3 ab	11.8 ab
		T5	27.2 a	11.2 a	69.4 a	54.6 a	11.7 b
		P (F)	**<0.001**	**<0.001**	**<0.001**	**0.002**	**<0.001**
		sem[b]	3.5	1.7	1.8	3.9	0.6
	Variety	Ketos	22.7 a	9.9 a	67.5 b	49.3 c	11.1 c
		Cometa	26.2 a	8.9 b	68.2 b	56.6 a	12.2 a
		Sfera	23.3 b	8.1 c	69.9 a	52.2 b	11.9 b
		P (F)	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
		sem[b]	2.7	1.4	1.4	3.0	0.5
		Treat x var	0.140	0.126	0.268	0.513	0.101
		sem[b]	6.4	3.0	3.2	6.8	1.1

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

[a] treatments: see table 1.

[b] sem = standard error of the mean

Table 6.

Effect of fungicide application on spot-type net blotch (STNB) incidence and severity and deoxynivalenol (DON) concentration; field experiments conducted in North West Italy in the 2010-2013 period.

Experiment	Factor	Source of variation	STNB Incidence[b]		STNB Severity[c]		DON	
			T	N (%)	T	N (%)	T	N (μg kg^{-1})
Cigliano 2010-2011	Treatment[a]	T2	84.1 a	97.2	32.8 a	29.7	4.0 a	73
		T5	44.6 b	49.2	7.5 b	1.9	4.9 a	190
		P (F)	**<0.001**		**<0.001**		0.057	
		sem[d]	14.9		7.2		0.6	
	Variety	Ketos	68.4 a	76.3	20.7 a	16.5	5.4 a	252
		Cometa	62.2 a	70.8	19.6 a	14.8	3.9 b	67
		Sfera	62.5 a	72.5	20.2 a	16.1	4.1 b	76
		P (F)	0.393		0.911		**0.030**	
		sem[d]	12.2		5.9		0.5	
		P (F)	0.806		0.962		0.220	
		sem[d]	21.1		10.2		0.8	
Cigliano 2011-2012	Treatment	T2	90.0 a	100.0	48.7 a	56.4	8.0 a	3683
		T5	42.5 b	45.8	7.7 b	1.9	6.7 b	1017
		P (F)	**<0.001**		**<0.001**		**0.001**	
		sem[d]	8.9		1.3		1.3	
	Variety	Ketos	65.8 a	73.8	29.2 a	30.3	7.6 a	3232
		Cometa	59.7 a	66.1	18.5 c	15.7	7.5 a	2140
		Sfera	51.5 b	62.4	20.9 b	24.1	6.9 a	1679
		P (F)	0.264		**<0.001**		0.322	
		sem[d]	7.3		1.1		1.1	
		P (F)	0.264		**0.001**		0.497	
		sem[d]	12.6		1.9		1.8	
Cigliano 2012-2013	Treatment	T2	90.0 a	100	44.5 a	49.1	5.7 a	449
		T5	53.7 b	61.7	9.1 b	2.8	4.8 b	182
		P (F)	**<0.001**		**<0.001**		**0.034**	
		sem[d]	14.8		4.9		2.3	
	Variety	Ketos	67.7 a	75.6	26.4 a	25.9	5.9 a	579
		Cometa	71.1 a	78.7	28.5 a	27.5	5.7 a	451
		Sfera	79.7 a	91.1	28.7 a	28.6	4.6 b	109
		P (F)	0.191		0.412		**0.002**	

		sem[d]	12.0		4.0		1.8		
		P (F)	0.191		0.544		0.266		
		sem[d]	20.9		6.9		3.2		
Cuneo 2012-2013	Treatment	T2	90.0 a	100	28.3 a	22.9	4.6 a	126	
		T5	85.8 a	98.3	13.7 b	6.0	3.6 b	51	
		P (F)	0.058		**<0.001**		**<0.001**		
		sem[d]	7.3		1.5		0.7		
	Variety	Ketos	88.5 a	99.5	18.5 a	10.9	5.0 a	118	
		Cometa	86.8 a	98.8	20.9 a	14.8	3.9 b	57	
		Sfera	88.1 a	99.2	22.4 a	16.1	3.2 c	32	
		P (F)	0.759		0.511		**<0.001**		
		sem[d]	5.9		1.2		0.6		
		P (F)	0.759		0.489		0.681		
		sem[d]	10.3		2.1		0.8		

Means followed by different letters are significantly different (the level of significance is shown in the table). Reported values are based on 4 replications. The reported STNB incidence and severity means are value transformed (T; y'=arcsen√x*180/π) and not transformed (N). The reported DON contamination means are value transformed [T; y'= ln (x + 1)] and not transformed (N).

[a] treatments: see table 1.

[b] STNB incidence was calculated as the percentage of leaves with symptoms of disease at the dough stage (GS 85), based on 4 replications of 2 leaves from 15 different plants.

[c] STNB severity was calculated as the percentage of leaf surface with symptoms of disease at the dough stage (GS 85), based on 4 replications of 2 leaves from 15 different plants.

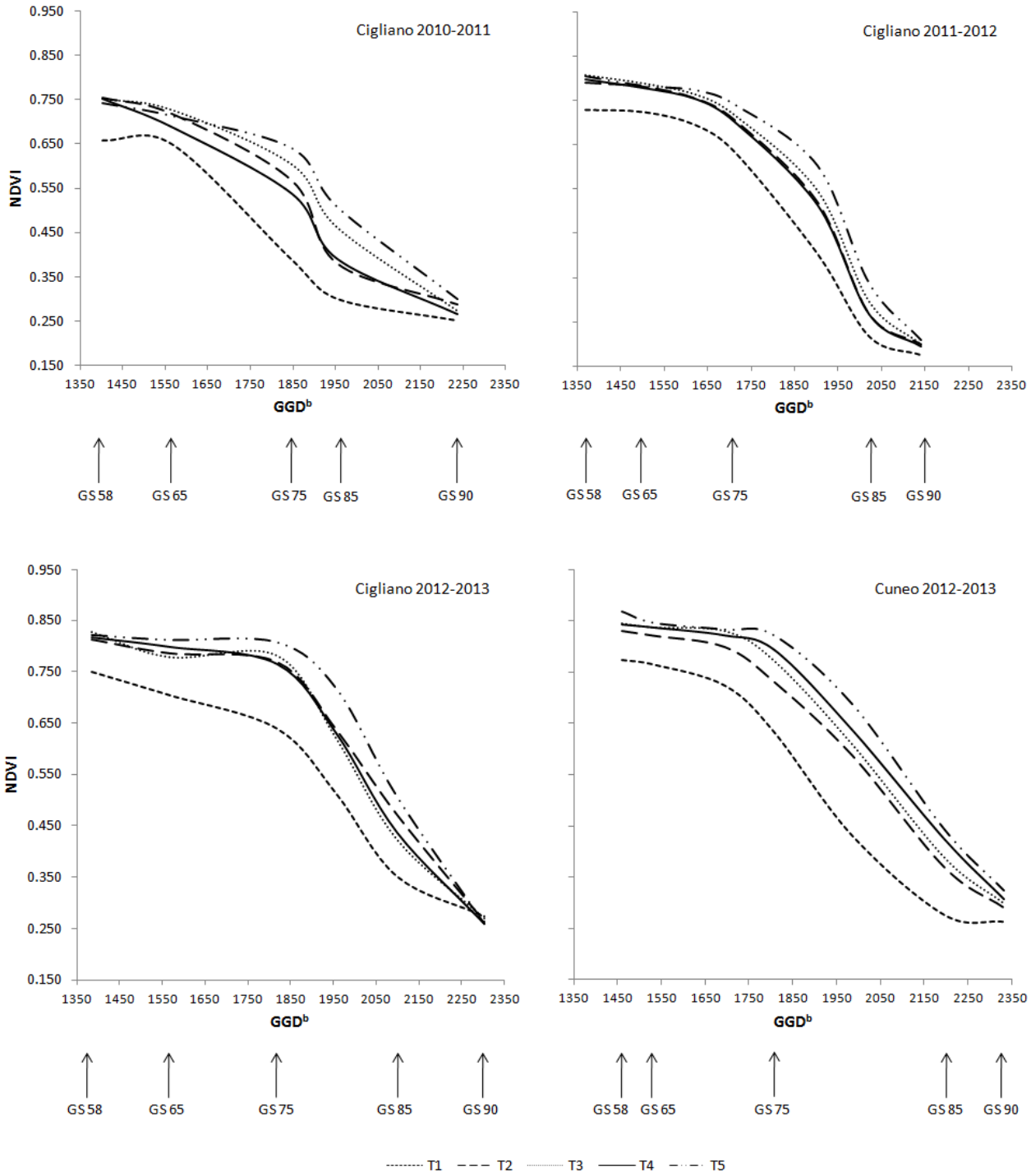
[d] sem = standard error of the mean

1

2

Figure 1.

Effect of foliar fertilization and fungicide protection on the canopy greenness (NDVI) of winter barley at different GS[a]; field experiments conducted in North West Italy in the 2010-2013 period.



[a] Growth stage (Zadocks et al., 1974).

[b] Accumulated growing degree days from sowing using a 0°C base .