Timing of azoxystrobin + propiconazole application on maize to control Northern Corn Leaf Blight and maximize grain yield.

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Timing of azoxystrobin + propiconazole application on maize to control northern corn leaf blight and maximize grain yield.

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Abstract

The use of foliar fungicides on field maize has increased greatly over the past ten years. There has also been an increasing interest in foliar fungicide applications on maize, because quinone outside inhibitor (QoI) fungicides, in addition to providing disease control, have been shown to induce physiological benefits for plants in studies conducted under controlled conditions. The aim of this research was to evaluate the effect of the timing of fungicide applications on maximizing grain yield by considering foliar disease control and physiological benefit of the application on plants. Five fungicide application timings were compared to an untreated control (T0), in four experimental trials conducted in 2 sites in 2009 and 2010. The fungicide treatments were applied in each trial at the following growth stages: T1, 4 unfolded leaves (growth stages - GS14); T2, end of leaf development (GS 19); T3, middle of stem elongation (GS 35); T4, flowering with fully emerged stigmata (GS 65); T5, milk stage (GS 75). The treatments were carried out with self-propelled ground sprayers, using a mixture of azoxystrobin and propiconazole. The following measurements were performed: plant and ear height, cross-sectional area of the stalk, leaf greenness, northern corn leaf blight (NCLB) incidence and severity, the photosynthetic efficiency of the total content of nonstructural carbohydrates of the leaf, ear dimension, grain yield, test weight (TW), thousand grain weight (TGW), European corn borer and fungal ear rot severity and fumonisin concentration in grain. Azoxystrobin + propiconazole application timing significantly affected NCLB incidence and severity, grain yield, TW and TGW. The best timings for foliar NCLB control were observed with application from the mid-stem elongation (T3) to the milk stage (T5), while only treatments at the mid-stem elongation (T3) and the flowering

stage (T4) significantly increased grain yield compared to the untreated control (T1). The first collected data suggest that plants treated with QoI and DMI fungicides undergo an increase in photosynthetic efficiency, while no significant differences have been observed for ear and plant development or leaf senescence for any application timings. None of compared fungicide application timings resulted in a significantly different concentration of fumonisins or severity of fungal ear rot than the untreated control.

Keywords: maize, fungicide, azoxystrobin, propiconazole, application timing, northern corn leaf blight

Abbreviations: ECB, European Corn Borer; GLS, gray leaf spot; GDDs, growing degree days; GS, growth stage; LNSC, leaf nonstructural carbohydrates; HNT, Hydro N-Tester; NCLB, northern corn leaf blight; PE, photosynthetic efficiency; RH, relative humidity; TW, test weight, TGW, thousand grain weight; WCRW, Western Corn Root Worm.

Introduction

The use of foliar fungicides on field maize has increased greatly over the past ten years in the United States (Munkvold et al., 2008), but also in other countries, such as Brazil (da Cunha et al., 2010) and Canada (Hooker et al. 2009), in an attempt to control foliar diseases and increase yields. In 2011, more than 4 million maize hectares were sprayed with foliar fungicide in the United States (Wise and Mueller, 2011).

Fungicides are usually applied using aerial equipment at maize flowering to control several foliar diseases, such as gray leaf spot (GLS), which is caused by *Cercospora zeae-maydis* (Ward et al., 1997a; Munkvold et al., 2001), common rust, caused by *Puccinia sorghi* (Pataky et al., 2002) and northern corn leaf blight (NCLB), caused by *Exserohilum turcicum* (Bowen and Pedersen, 1988; Da Costa and Boller, 2008) and others.

NCLB is the predominant foliar disease In North Italy. The disease appears as long, elliptical grey-green or tan streaks. As the disease develops, individual lesions may join up to form large blighted areas. Losses due to NCLB are more severe when the leaves above the ear are infected at, or slightly after, flowering. Humid and warm weather, late planting and an abundance of previous maize residues increase the risk of disease infections (Munkvold and Gorman, 2006).

Although the use of fungicides is not always consistently profitable for maize, Paul et al. (2011), through a random–effect meta-analyses on 212 studies conducted from 2002 to 2009 in the United States, demonstrated that the mean yield difference between the untreated control and a foliar fungicide application between tassel emergence [Growth stage (GS) 51, Lancashire et al., 1991; Weber and Bleiholder, 1990] and silk emergence (GS 65) was positive and significantly different from zero.

Profitable fungicide use in maize depends to a great extent on the grain yield potential, the disease-susceptibility of the planted hybrid and the foliar disease severity throughout the growing season, the market price of maize and the price for fungicide application.

Demethylation inhibitor (DMI) fungicides, and particularly propiconazole, have shown the highest efficacy in controlling NCLB both *in vitro* and in field conditions (Bowen and Pedersen, 1988; Kumar et al., 2009). Moreover, there is an increasing interest in foliar fungicide applications to maize for reasons other than simple disease control (Munkvold et al., 2008). In particular, the quinine outside inhibitor (QoI) fungicides, commonly referred as strobilurin, in addition to providing disease control, have been shown to induce physiological benefits for plants, including improved stalk strength, longer preserved green leaf tissue and delayed plant senescence (Wu and von Tiedemann, 2001), either through a reduction in ethylene or in oxidative stress (Grossman and Retzlaff, 1997; Zhang et al., 2010), an increase in photosynthetic capacity and translocation (Gooding et al., 2000) and regulation of the stomatal aperture and improved water-use efficiency (Grossman et al., 1999).

Studying the timing of fungicide treatments for the GLS management of maize, Ward et al. (1997b), reported that the yield response to fungicides appeared to be a function of the plant growth stage, the amount of disease at the spray date and of the effective control through to physiological maturity. In order to control foliar diseases, fungicide applications in the U.S. Corn Belt are generally suggested at maize flowering (Nelson and Meinhardt, 2011). However, the best fungicide application timing for maize needs to be better understood in order to maximize grain yield, considering not only foliar disease control but also the additional physiological benefit for plants related to the use of a mixture of QoIs and DMI fungicides.

Moreover, since the yield response to fungicide applications is not always consistently profitable for maize, there is some interest in reducing the application costs, by applying the fungicides at different times and tank-mixing them with other products, such as post-emergence herbicides or insecticides.

Fungicide applications in the early vegetative growth stages of maize (GS13-15), together with the post-emergence herbicides, could preventively protect maize against disease infection (Pataky et al., 2002). Moreover, since maize ear initiation begins at these growth stages (Lejeune and Bernier, 1996), the fungicide could enhance some plant performances, such as the number of kernels per row and the number of kernel rows per ear. Furthermore, the use of chemical insecticides is the main method used to manage European Corn Borer (ECB, *Ostrinia nubilalis* Hübner) and Western Corn RootWorm (WCRW, *Diabrotica virgifera virgifera* LeConte), where Bt maize cultivation is not permitted (Saladini et al., 2009). In North Italy, treatments are carried out successfully with specific self-propelled sprayers that are able to ground spray the maize crop during the ripening stages (Blandino et al., 2009). Fungicide applications in mixtures with insecticides could control foliar disease more efficiently during the last part of ripening and preserve the green leaf tissue longer, by providing a “greening” effect.

The potential effect of these fungicide treatments on fungal ear rot diseases and mycotoxin contamination still needs to be verified. Fungicide applications, in particular those from flowering to the ripening GSs, could play a role in reducing *Fusarium verticillioides* and fumonisin concentration in maize kernels (Mazzoni et al., 2011). On the other hand, the fungicide action could also influence the different fungal species, by changing the ratio between the toxigenic and non toxigenic fungi. It is well known, for example, that a QoI application at wheat heading could increase

fusarium-toxin contamination, since it reduces the infection of species that are not able to synthesise mycotoxins, while it increases the infection of the toxic *Fusarium* species (Pirgozliev et al., 2003). Therefore, when fungicides are applied to cereal crops, their implications on mycotoxin production should be considered.

The aim of this research was to evaluate the effect of the timing of fungicide application with a mixture of azoxystrobin + propiconazole on NCLB incidence and severity and grain yield in maize cultivated in NW Italy, in relation to several plant performance characteristics. The consequences of the application of this fungicide mixture on fungal ear rot and fumonisin contamination were also assessed.

Materials and methods

Experimental site and treatments

The effect of fungicide application on the control of NCLB and maize yield was studied in 2009 and 2010 in 2 sites in NW Italy: site A (Saluggia, 45° 14' N, 8° 00' E; altitude of 194 m., in a shallow and sandy soil, Typic Hapludalfs, according to the USDA classification) and site B (Villafranca P.te, 44° 47' N, 7° 33' E altitude of 253 m., in a deep and fertile sandy soil, Typic Eutrochrepts, according to the USDA classification).

Five fungicide application timings (T1-T5) were compared to an untreated control (T0), in each site and in each year. The fungicide treatments were applied in each trial once at the following growth stages (GS, Weber and Bleiholder, 1990; Lancashire et al., 1991), according to the development of the untreated control:

- T1: leaf development at 4 unfolded leaves (GS 14), according to the correct application timing for a post-emergence weed control treatment;
- T2: end of leaf development (GS 19), at the feasible height limit to allow entrance with a common farm ground sprayer;
- T3: middle of stem elongation with 5 detectable nodes (GS 35);
- T4: flowering with fully emerged stigmata (GS 65);
- T5: milk stage (GS 75), according to the best application timing in North Italy for an insecticide application to control European corn borer (ECB), which is based on adult insect captures (Blandino et al., 2009).

The sowing, silking and harvest dates and the fungicide application dates are reported in table 1, for each year and site.

The applied fungicide was a mixture of QoI and DMI fungicide: azoxystrobin and propiconazole (Quilt Xcel™), formulation: emulsifiable concentrate (EC), Syngenta Crop Protection AG, Basel, Switzerland) and was applied at 0.141 and 0.122 kg AI ha^{-1}, respectively (1 L of commercial product ha^{-1}). Treatments were carried out using self-propelled ground sprayers (Eurofalcon E140®, Finotto), with a hydraulically adjustable working height (0.40 – 4.30 m) in order to spray the maize crop also after flowering. Twenty flat-fan nozzles on the air-assisted boom applied a spray volume of 400 l ha^{-1} at a pressure of 200 kPa and a median droplet size range of 145-225 microns; the operation speed was 10 km h^{-1}. Air-assisted spraying uses relatively large volumes of low-pressure air, which is generated by a fan, to direct the spray onto the crop.

The treatments were assigned to experimental units using a randomized complete block design with 4 replicates. Each plot consisted of 16 rows 0.75 m apart, separated by two untreated buffer rows on either side; the plot length and the alleys between the plots were 25 and 1 m, respectively.

Studies were carried out each year on the commercial dent corn hybrid Syngenta NX7034 (FAO maturity class 600; 128 days relative to maturity), with medium susceptibility to NCLB. The previous crop was maize each year. Planting was carried out after an autumn ploughing to a 30 cm depth, thus incorporating the debris in the soil, followed by disk harrowing to prepare a proper seedbed.

The experiment fields received 250, 100 and 100 kg ha^{-1} of N, P_2O_5 and K_2O respectively each year and applied at both site. Irrigation was applied in both site using the furrow surface method to maintain the water-holding capacity at between 33 and 200 kPa. Weed control was conducted at pre-emergence with mesotrione (0.15 kg AI ha^{-1}) S-metolachlor (1.25 kg AI ha^{-1}) and terbuthylazine (0.75 kg AI ha^{-1})

(Lumax®, Syngenta Crop Protection S.p.A., Milan, Italy). All the trials were treated with insecticide at GS 75; the insecticide was pyrethroid lambda-cyhalothrin (Karate® Zeon, Syngenta Crop Protection S.p.A., Milan, Italy) and it was applied at 0.019 kg AI ha^{-1}.

Crop measurements and analysis

During the crop maturation stage, the following measurements were performed on 15 plants from each plot by randomly selection of 3 sub-plots of 1 m of row each in the middle 10 rows: NCLB incidence and severity, plant and ear height, cross-sectional area of the stalk and leaf greenness.

Fifteen plants were visually evaluated at flowering (GS 65), the milk stage (GS 75) and dough stage (GS 85) in each plot to establish the incidence and severity of the NCLB symptoms. At GS 65 and GS 75, the disease evaluation was conducted in all plots just before of the fungicide application for T4 and T5 treatments, respectively. Five leaves were considered for each plant: the ear leaf and the 2 leaves above and under the ear. The NCLB incidence was calculated as the percentage of leaves with symptoms (considering 75 leaves per plot), while the NCLB severity was calculated as the average percentage of leaf surface with symptoms. A scale of 1 to 7 was used in which each numerical value corresponds to a percentage interval of foliar surfaces exhibiting visible symptoms according to the following schedule: 1 = no symptoms, 2 = 1-2%, 3 = 3-5%; 4 = 6-10 %, 5 = 10-25%, 6 = 26-50%, 7 > 50%. The NCLB severity scores were converted into percentages of leaves exhibiting symptoms and each score was replaced with the mid-point of the interval.

The plant and ear height and cross-sectional areas of the stalks were recorded at GS

85. Plant height was measured in centimeters from the ground level up to the base of the flag leaf. Ear height was measured as the number of centimeters from the ground level up to ear insertion. The cross-sectional area of the stalk was calculated from the stalk diameters between the first and second nodes and it was measured using a 0.1 mm caliper.

Leaf greenness was estimated at GS 75 and GS 85. A chlorophyll meter, Hydro N-Tester® (HNT) (Hydro-Agri, now Yara, Yara Italia SpA, Milan, Italy) was used to measure the relative leaf greenness. The HNT values are numerical, dimensionless values that are proportional to the amount of total chlorophyll present in the leaf (Arregui et al., 2006). Readings were taken using the HNT at mid-length of the ear leaf and the leaf above the ear.

In the 2010 trials, leaf samples (ear leaf and the leaf above the ear) were collected from 7 plants from each plot of the T0, T3 and T4 treatments to determine the total content of leaf nonstructural carbohydrates (LNSC). In both sites, leaf sampling was performed by hand clipping at the leaf base at sunset (July 25) and at sunrise of the following day (July 26). The leaf samples were immediately frozen at -18°C, then, after lyophilization, the tissue was ground in a cyclone sample mill to pass a 1.0 mm screen. The leaf tissue collected from plots referring to the same treatment in each site were analyzed together. The ground material was mixed thoroughly prior to the LNSC analyses. The LNSC analysis was performed as described by Kerr et al., (1985), with an ethanol (80% v/v) extraction for 10 min at 80°C, followed by spectrophotometric quantification. LNSC was expressed as milligram per gram dry weight. The relation between the diurnal accumulation of LNSC and nocturnal utilization can be expressed using parameter the photosynthetic efficiency (PE) parameter, which is defined by the following ratio:

$$PE\ (\%) = \left(\frac{\text{LNSC sunset} - \text{LNSC sunrise}}{\text{LNSC sunset}} \right) \text{x } 100$$

The ears were collected by hand from 15 m^2 (4 rows X 5 m) in each plot at the end of maturity, at a grain moisture content of between 23-26%. A sub-sample of 45 ears was used to evaluate ECB and fungal ear rot severity and the ear dimensions, after removing the husk. The ECB damage severity was calculated as the percentage of kernels per ear with injuries due to larvae activity. A scale of 1 to 7 was used in which each numerical value corresponds to a percentage interval of surfaces exhibiting visible kernel damage due to larvae activity according to the following schedule: 1 = no injuries, 2 = 1-5%, 3 = 6-10%; 4 = 11-20 %, 5 = 21-35%, 6 = 35-60%, 7 > 60% (Blandino et al., 2009). The fungal ear rot severity was calculated 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 = no symptoms, 2 = 1-3 %, 3 = 4-10%; 4 = 11-25 %, 5 = 26-50%, 6 = 51-75%, 7 > 75% (Blandino et al. 2009). The ECB damage severity and ear rot severity scores were converted to percentages of ears exhibiting symptoms and each score was replaced with the mid-point of the interval. The ear length, the number of kernels per row and the number of kernel rows per ear were measured on 45 de-husked ears from each plot. Data for the number of kernels per row and the number of kernel rows per ear were only recorded in the 2010 trials.

All the collected ears were shelled using an electric sheller to obtain grain weight. The grain yield results were adjusted to a 140 g kg^{-1} moisture content. The kernels in each plot were mixed thoroughly to obtain a random distribution and 1 kg was taken

to measure the moisture content, test weight (TW) and thousand grain weight (TGW), while 5 kg samples were taken to analyze the fumonisin (FB_1 and FB_2) concentration. The moisture concentration of the wet maize grain and the test weight of the dried grain were determined by means of a Dickey-John GAC2000 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 balance to assess the TGW.

A 5 kg representative sample of grain from each plot was freeze-dried and milled. A 50 g representative sub-sample of the milled material was analyzed for toxin concentration. The fumonisin B_1 and B_2 contaminations were analyzed according to the method proposed by Visconti et al. (2001). Fumonisins were extracted from 10 g samples in a plastic centrifuge bottle with 50 ml of acetonitrile:methanol:water (25:25:50, v/v/v). After extraction for 45 min, using a rotary-shaking stirrer and centrifugation at 4500 g for 6 min, the supernatant was poured into a flask; another 50 ml of the same solution was added to the residue in the centrifuge bottle, and a second extraction performed for 30 min. The combined extracts were filtered through a folded filter-paper. Analysis was carried out using an LC-MS/MS system. The limit of detection (LOD) of the analytical method was 10 $\mu g\ kg^{-1}$ for fumonisin B_1 and FB_2.

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 plant and ear height, cross-sectional area of the stalk, HNT readings, grain yield, grain moisture, TGW, TW, ear length, number of kernels per row, kernel rows per ear, ECB and fungal severity, and the fumonisin B_1 + B_2 concentrations, using a completely randomized block design, in which the timing of the fungicide application was the independent variable and the trial (different years and sites) was the random factor. ANOVA was utilized to separately compare NCLB incidence and severity, for all the year and site combinations, using a completely randomized block design, in which the timing of the fungicide application was the independent variable. Multiple comparison tests were performed according to the Ryan-Einot-Gabriel-Welsch (REGW) test on the treatment means. SPSS Version 16.0 for Windows statistical package, (SPSS Inc., 2008), was used for the statistical analysis.

The incidence and the severity values of NCLB, the severity of ECB and fungal ear rot had previously been transformed using $y'=\arcsin\sqrt{x}*180/\pi$, as percentage data derived from counting. The concentration of fumonisins was transformed using the equation $y'=\ln(x+1)$ to normalize the residuals.

Results

The May-October period in the two experiment sites had similar meteorological trends each year, for rainfall, relative humidity (RH) and temperature (expressed as growing degree days, GDDs) from flowering to harvest (Table 2). There was more frequent rainfall in 2010 than in 2009 in both sites, with a higher average RH but lower temperatures, particularly from the beginning of the stem elongation to flowering (June) and during the dough stages (August and September).

NCLB incidence and severity

The first symptoms of NCLB were noticed at maize flowering (GS65), in both years. However, the disease symptoms from flowering (GS 65) to the milk stage (GS 75) were very low: in all trials and for all treatments the NCLB incidence was lower than 2% (data not shown). ANOVA did not show a significant effect of the fungicide application timing on NCLB incidence and severity at maize flowering and at milk stage.

The NCLB symptoms at the dough stage were clearly affected by the year: the disease incidence and severity were higher in 2010 than in 2009 in both sites, as a consequence of the greater rainfall, RH and higher temperatures that occurred, especially during the ripening stages.

As reported in figures 1 and 2, ANOVA showed a significant effect ($P<0.05$) of the azoxystrobin + propiconazole application timings on NCLB incidence and severity.

The disease symptoms generally decreased moving from the earlier application timings to the later ones.

In 2009, at site A, NCLB incidence was reduced significantly by 73% in the T4 and T5 treatments compared to T0, while, at site B, the T3, T4 and T5 application timings showed a significantly lower incidence than T0. At site A, only the T5 treatment showed a significantly lower NCLB severity than T0. At site B, the application from the stem elongation stage (T3) to the milk stage (T5) significantly differed as far as disease severity is concerned compared to the untreated control (T0). The NCLB severity reduction, compared to T0 was 85, 92 and 93% for T3, T4 and T5, respectively.

In 2010, in both sites, the azoxystrobin + propiconazole application at the T3, T4 and T5 timings significantly reduced disease incidence and severity compared to T0.

The NCLB incidence and severity for T1 and T2 was never significantly different from those observed in the untreated control (T0) in any of the trials.

Crop measurements

ANOVA did not show a significant effect of the fungicide application timing on plant and ear height, cross-sectional area of the stalk or leaf greenness, evaluated through HNT reading at GS 75 and 85 (Table 3). No significant effects of the interaction between the fungicide treatments and trials were observed.

In 2010, in both sites, the photosynthetic efficiency (PE), based on the nonstructural carbohydrates in the maize leaf at sunset and at sunrise, was significantly higher in T3 treatment compared to T0 (Fig. 3). Among the compared treatments, the fungicide application at the mid-stem elongation stage (T3) showed the highest PE, which was 72% and 68% for site A and B, respectively. The PE of the T4 treatment was 65%

and 62% for site A and B, respectively and was not significantly different compared to that recorded for T3 treatment.

Yield parameters and ear dimensions

A significant effect ($P<0.001$) of the azoxystrobin + propiconazole application timing on maize grain yield, TGW and TW was observed (Table 4).

The T1, T2 and T5 treatments were not significantly different from the untreated control (T0). The azoxystrobin + propiconazole application at the mid-stem elongation (T3) and at the flowering stage (T4) significantly increased grain yield (+5%; 0.75 t ha^{-1}), TGW (+ 11.6 g) and TW (+ 0.58 kg hl^{-1}), compared to T0. No significant differences were observed between the T3 and T4 treatments for any of the previous parameters.

No significant differences were observed for the grain moisture content, ear length, number of kernels per row and kernel rows per ear between the different azoxystrobin + propiconazole application timings and the untreated control.

The interactions between the independent variable (fungicide treatment) and random factor (trial) were never significant.

ECB and fungal ear rot severity and fumonisin contamination

ANOVA did not show a significant effect of the azoxystrobin + propiconazole application on ECB severity, fungal ear rot severity or fumonisin B_1 + B_2 contamination (Table 5). The interactions between the independent variable (fungicide treatment) and random factor (trial) were never significant.

Discussion

The data have clearly shown the positive effect of azoxystrobin + propiconazole application on NCLB control and maize grain yield. Moreover, the collected data show that the efficacy of the fungicide is closely related to the application timing.

In the trials with higher disease pressure (2010), the NLCB severity at the dough stage was significantly reduced through a fungicide application from the middle of stem elongation to the milk stage. Instead, with the lower disease pressure observed in the 2009 trials, only the applications at flowering (site B) and at the milk stage (site A and B) resulted in a significant lower NCLB severity than the untreated control. These results suggest that, considering only one fungicide application, the best disease control during maize ripening could be achieved by starting the application at flowering, confirming the common recommendation for foliar disease control through fungicide application to maize (Munkvold and Gorman, 2006). In order to maximize grain yield through foliar fungicide application, Ward et al., (1997b) demonstrated the importance of controlling disease during the ripening stage through to physiological maturity.

Our results have therefore reported that there are differences in grain yield advantage between the fungicide application timings which provide a significant control of NCLB at dough stage. In fact, the fungicide application at the milk stage (T5) did not significantly increase grain yield compared to the untreated control, although the NCLB symptoms from flowering to dough stage for T3, T4 and T5 treatment were not significantly different. On the other hand, since no interaction exists between the treatment timings and the combination of site and year, the fungicide application between the mid-stem elongation and the maize flowering stage led to a significant and comparable increase in grain yield in all the trials, even for a

low foliar disease severity in the untreated control. These data suggest that the potential yield benefit for maize crops sprayed with a mixture of QoI and DMI fungicide could be related to physiological effect on plants and not only a consequence of disease control. However, fungicides may affect grain yield by controlling minor fungal pathogens that are not easy to identify in the field. Bertelsen et al., (2001) reported that yield increases obtained in wheat by azoxystrobin and epoxiconazole application to field with very low levels of visible disease could be due to control of saprophytic fungi and a lower energy cost for defence reactions. Nevertheless, in the wheat experiment, grain yield increase due to the control of the saprophytes was strongly related to a delay in plant senescence. In our experiments, no noticeable differences in maize plant senescence among treatments were observed.

QoI-containing fungicides have been shown to provide physiological benefits and increase grain yield through enhanced plant performance, even in the absence of disease in maize (Nelson and Meinhardt, 2011;) or in other crops (Bertelsen et al., 2001; Kato et al., 2011). In other experiments (Swoboda and Pedersen, 2009; Weisz et al., 2011), QoI-containing fungicides did not produce any physiological effects or associated yield improvement without significant disease pressure. Most of data reported on the effect of QoI -containing fungicide on maize grain yield considered only treatments applied between tasseling and the first ripening stages (Paul et al., 2011). In our experiments, the highest grain yield have been achieved with the fungicide application at mid-stem elongation stage.

The data reported in the present study have shown that the yield-enhancing effects obtained from the fungicide application, are not related to a clear delay in plant senescence or to a change in plant or ear traits and development. No noticeable

abiotic stresses affected the crops in any of the trials. The results of Bradley and Ames (2010) indicated that QoI-containing fungicides provided very little benefit to maize injured by simulated hail, in terms of grain yield, although it reduced GLS severity, which was increased by hail damage.

The application of pyraclostrobin, a QoI fungicides, have been reported to decrease ethylene levels on maize, although this effect could lead to risk of injury (Below et al., 2009). In this experiment, the magnitude of an ear abnormality called hollow husk, normal appearing husks that feel hollow due to an abrupt cessation in ear development and a lack of silk emergence, was intensified by the application of this fungicide during the vegetative stages. Plants sprayed at GS35 exhibited greater symptoms than those sprayed at GS19, suggesting a higher physiological effect in reducing plant ethylene levels with the latest timing application.

In the present manuscript, the fungicide application timings that lead to the highest grain yield, also resulted in a significant increase in TGW, compared to the untreated control. Thus, the main physiological effect could be related to a better photosynthetic efficiency of the plant. Treatments with pyraclostrobin significantly increased TGW in soybean compared to other fungicides, although the efficacy in reducing leaf diseases was similar (Kato et al., 2011).

Our data referring to the LNSC diurnal accumulation and nocturnal utilization, although of a preliminary nature, seem to suggest a higher capacity to produce and translocate the photoassimilates of fungicide-treated leaves. It is well known that soluble carbohydrate levels are higher in diseased leaves than in healthy leaves (Wright et al., 1995). Soybean plants treated with QoIs showed an increase in the net carbon assimilation rate, which led to a higher TGW and grain yield (Fagan et al., 2010). A higher photosynthetic activity of QoI -treated plants was also observed in

wheat, in the absence of foliar diseases through gas exchange and chlorophyll fluorescence measurements in field conditions (Beck et al., 2002). Furthermore, QoI-containing fungicides are known inhibitors of mitochondrial respiration in plants (Venancio et at., 2003), and Nason et al. (2007) reported that QoI-containing fungicides reduce dark respiration in wheat leaves. Since maize crops show the highest photosynthetic rate between stem elongation and the flowering (Ding et al., 2007), these stages could maximize the physiological benefit of the QoI-containing fungicide application.

The present study has shown that early and late fungicide applications, when mixed with post-emergence herbicides or with insecticides to control ECB or NWRW to reduce application costs, did not significantly increase grain yield compared to an untreated control. The azoxystrobin + propiconazole application at the leaf emission stages resulted in no difference in NCLB incidence and severity, plant and ear height, stalk section area, or ear traits with the untreated control. Application at the milk stage did not lead to a clear delay of plant senescence compared to the untreated plot, although it resulted in a significantly lower foliar disease incidence and severity at dough stage.

As far as the mycotoxin contamination of grain is concerned, none of compared fungicide application timings resulted in a significantly different concentration of fumonisins or severity of fungal ear rot than untreated control. Although infection through silks is a significant source of infection by *F. verticillioides* (Munkvold et al., 1997), field trials conducted to assess the effect of fungicides applied at maize flowering on ear rot and fumonisin control have yielded conflicting results. In non inoculated field experiments in North Italy, where ECB infestation was controlled through insecticide applications, several mixtures of DMI fungicides applied at maize

flowering were able to reduce maize silk colonization by *F. verticillioides* (Causin et al., 2008). However, Folcher et al. (2009) and Mazzoni et al. (2010) reported that the addition of an DMI fungicide (tebuconazole or tebuconazole + prothioconazole) to an insecticide treatment at flowering did not significantly reduce the fumonisin concentration in maize kernels compared to an insecticide application alone. Moreover, the data reported in the present study did not show any synergy or negative effects on controlling the fumonisin concentration when a fungicide, a combination of QoI and DMI active ingredient, was sprayed at the milk stage together with a correct insecticide application. Other researches have reported that *Fusarium* spp. could have a low sensitivity to QoI fungicides (Broders et al., 2007; Gutierrez Chapin et al., 2006).

In conclusion, this research, which is to the authors knowledge the first in Europe, offers a further contribution towards determining the role of foliar fungicide applications in maize. The collected data underline that a mixture of QoI and DMI fungicide, applied between the mid-stem elongation and the flowering stage significantly increases grain yield and TGW. Since yield advantages have been observed even with low levels of NCLB severity, without significant interaction with the combination of site and year, and since the application at milk stage did not affect grain yield, although it provided the same control of foliar diseases observed for T3 and T4 treatments, these effects could be related to reasons other than simple disease control and could involve plant performance benefits. The preliminary data that has collected suggest that plants treated with QoI and DMI fungicides result in an increase in photosynthetic efficiency, while no significant differences have been observed for ear and plant development or leaf senescence. More research, based on specific physiological research programmes is needed to better understand the

physiological benefit of these products. Moreover, the yield-enhancing effects of foliar fungicides need to be verified in different maize cropping areas, in order to confirm this grain yield response in different agricultural and environmental conditions and to verify whether their use leads to a positive and sustainable economic benefit for maize growers. The probability of a profitable fungicide application is linked of the yield difference between treated and untreated plots, which is affected by yield potential, the cost associated with the application method and the product cost, as well as the market price of maize. Considering the maize grain yield and the fungicide treatment costs at the farm conditions of the current manuscript, the fungicide application at T3 and T4 timings results profitable with a minimum price of maize grains of 120 € t^{-1}.

Finally, it is important to recall that QoI -containing fungicides are very risky as far as resistance development is concerned, since applications of these fungicides can increase the selection pressure and this could lead to shifts in fungal sensitivity to QoI fungicides (Walker et al., 2009). Therefore, a better understanding of the impact of these fungicides in maize production is also urgently needed to maintain the long-term efficacy of these compounds.

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Tables

Table. 1.

Main trial information and date of fungicide application in the field experiments conducted in the 2009-2010 period.

Year	**2009**		**2010**	
Site	**A**	**B**	**A**	**B**
Treatment[z]		**(date of fungicide application)**		
T1	May 5	May 11	May 18	May 20
T2	June 6	June 6	June 4	June 11
T3	June 24	June 18	June 23	June 28
T4	July 2	July 3	July 5	July 7
T5	July 30	July 25	July 27	July 26
Maize growth stages				
Sowing date	April 22	April 9	April 9	April 1
Silking date	July 2	July 1	July 2	July 6
Harvest date	Sept. 28	Octob. 1	Sept. 21	Sept. 30

[z] Treatment: T1, application at leaf development at 4 unfolded leaves (GS 14); T2, application at end of leaf development (GS 19); T3, application at middle of stem elongation with 5 detectable nodes (GS 35); T4, application at flowering with fully emerged stigmata (GS 65); T5, application at milk stage (GS 75).

Table 2.

Total rainfall, rainy days, relative humidity and growing degree days (GDD 10s) from May to October 2009-2010 in the experimental sites of Saluggia (site A) and Villafranca P.te (site B).

Year	Site	A					B			
	Month	Rainfall (mm)	Rainy days (no.)	RH[y] (%)	GDD 10s[z] (°C d^{-1})		Rainfall (mm)	Rainy days (no.)	RH[y] (%)	GDD 10s[z] (°C d^{-1})
2009	May	43	8	65	294		66	6	62	281
	June	62	11	65	334		114	9	68	325
	July	35	5	66	397		24	9	69	391
	August	56	12	73	418		43	9	71	409
	September	188	12	75	284		81	11	74	264
	October	54	10	79	156		32	9	76	161
	May-October	438	58	71	1882	0	359	53	70	1831
2010	May	154	17	71	202		182	22	71	205
	June	141	13	71	328		146	13	71	310
	July	44	9	69	436		37	6	68	423
	August	180	12	75	351		89	10	74	342
	September	58	9	76	239		55	11	76	235
	October	151	17	82	113		119	15	82	114
	May-October	728	77	74	1668	0	627	77	74	1629

[Y] RH, Relative humidity

[z] GDD, Accumulated growing degree day for each month.

Table 3.

Effect of the azoxystrobin + propiconazole application timing on plant and height, cross-sectional area of stalk and Hydro N-tester (HNT) readings at the milk (GS 75) and dough stages (GS 85), field experiments conducted in 2 sites in the 2009 - 2010 period.

Factor	Source of variation	Plant height (cm)	Ear height (cm)	Cross-sectional area of stalk (cm^2)	N-tester reading	
					GS 75 (HNT unit)	GS 85 (HNT unit)
Trial	Site A, 2009	317	132	4.8	762	684
	Site B, 2009	307	129	5.0	690	590
	Site A, 2010	297	137	5.2	796	607
	Site B, 2010	314	163	5.2	778	683
Treatment[Y]	T0	307 a	142 a	5.1 a	753 a	636 a
	T1	308 a	140 a	5.0 a	765 a	629 a
	T2	308 a	141 a	5.1 a	764 a	642 a
	T3	308 a	139 a	5.1 a	761 a	644 a
	T4	309 a	140 a	5.1 a	760 a	653 a
	T5	307 a	139 a	5.1 a	761 a	645 a
	P (F)	0.878	0.326	0.929	0.478	0.264
	sem[Z]	6.3	2.4	0.20	18.1	29.2
Treatment X Trial	*P* (F)	0.996	0.998	0.997	0.675	0.955

Means followed by different letters are significantly different (the level of significance is shown in the table). The reported values of the trial factor are based on 24 replications (6 treatment X 4 repetitions), while the values of the treatment factor are based on 16 replications (4 trials X 4 repetitions).

[Y] Treatment: T0, untreated control; T1, fungicide application at leaf development at 4 unfolded leaves (GS 14); T2, fungicide application at end of leaf development (GS 19); T3, fungicide application at middle of stem elongation with 5 detectable nodes (GS 35); T4, fungicide application at flowering with fully emerged stigmata (GS 65); T5, fungicide application at milk stage (GS 75).

[Z] sem: standard error of mean.

Table 4.

Effect of the azoxystrobin + propiconazole application timing on grain yield, moisture content at harvest, thousand grain weight (TGW), test weight (TW), ear length, kernels per row and kernel rows per ear; field experiments conducted in 2 sites in the 2009 - 2010 period.

Factor	Source of variation	Grain yield (t ha^{-1})	Grain moisture (%)	TGW (g)	TW (kg hl^{-1})	Ear lenght (cm)	Kernels per row (N°)	Kernel rows per ear (N°)
Trial	Site A, 2009	13.7	23.4	396	76.7	19.2	n.p.	n.p.
	Site B, 2009	14.2	25.4	381	76.3	19.9	n.p.	n.p.
	Site A, 2010	15.3	25.0	400	75.8	19.2	38.3	16.4
	Site B, 2010	15.4	25.7	396	75.8	20.1	39.2	16.0
Treatment[Y]	T0	14.4 c	24.6 a	389 c	75.8 c	19.5 a	38.4 a	16.2 a
	T1	14.4 c	24.7 a	389 c	75.8 bc	19.4 a	38.4 a	16.1 a
	T2	14.7 bc	25.0 a	395 abc	76.2 abc	19.7 a	38.7 a	16.1 a
	T3	15.3 a	24.9 a	403 a	76.5 a	19.7 a	39.3 a	16.2 a
	T4	15.1 ab	25.1 a	398 ab	76.2 ab	19.7 a	39.3 a	16.2 a
	T5	14.5 c	25.2 a	392 bc	76.0 bc	19.5 a	38.2 a	16.3 a
	P (F)	< 0.001***	0.097	< 0.001***	< 0.001***	0.127	0.071	0.808
	sem[Z]	0.20	0.47	6.66	0.28	0.36	0.38	0.13
Treatment X Trial	*P* (F)	0.960	0.969	0.654	0.947	0.986	0.795	0.944

Means followed by different letters are significantly different (the level of significance is shown in the table). The reported values of the trial factor are based on 24 replications (6 treatment X 4 repetitions), while the values of the treatment factor are based on 16 replications (4 trials X 4 repetitions).

[Y] Treatment: T0, untreated control; T1, fungicide application at leaf development at 4 unfolded leaves (GS 14); T2, fungicide application at end of leaf development (GS 19); T3, fungicide application at middle of stem elongation with 5 detectable nodes (GS 35); T4, fungicide application at flowering with fully emerged stigmata (GS 65); T5, fungicide application at milk stage (GS 75).

[Z] sem: standard error of mean

n.p. measurements not performed

Table 5.

Effect of the azoxystrobin + propiconazole application timing on ECB and fungal ear rot severity and fumonisin $B_1 + B_2$ contamination; field experiments conducted in 2 sites in the 2009 - 2010 period.

Factor	Source of variation	ECB severity		Fungal ear rot severity		Fumonisin $B_1 + B_2$	
		T	T (%)	T	T (%)	T	N (µg kg^{-1})
Trial	Site A, 2009	8.5	2.4	8.7	2.6	5.7	359
	Site B, 2009	10.2	3.4	8.5	2.3	5.0	204
	Site A, 2010	11.2	4.1	9.6	2.9	7.5	1864
	Site B, 2010	13.2	5.5	9.7	3.3	7.1	1327
Treatment[Y]	T0	10.9 a	3.9	9.2 a	2.7	6.3 a	925
	T1	11.5 a	4.2	9.2 a	2.8	6.4 a	922
	T2	10.4 a	3.7	9.4 a	2.9	6.5 a	1020
	T3	10.7 a	3.8	8.8 a	2.5	6.6 a	1227
	T4	10.9 a	3.9	9.4 a	2.9	6.6 a	1199
	T5	9.4 a	2.9	8.7 a	2.6	6.5 a	902
	P (F)	0.320		0.950		0.851	
	sem[Z]	2.23		1.90		0.60	
Treatment X Trial	*P* (F)	0.956		0.978		0.264	

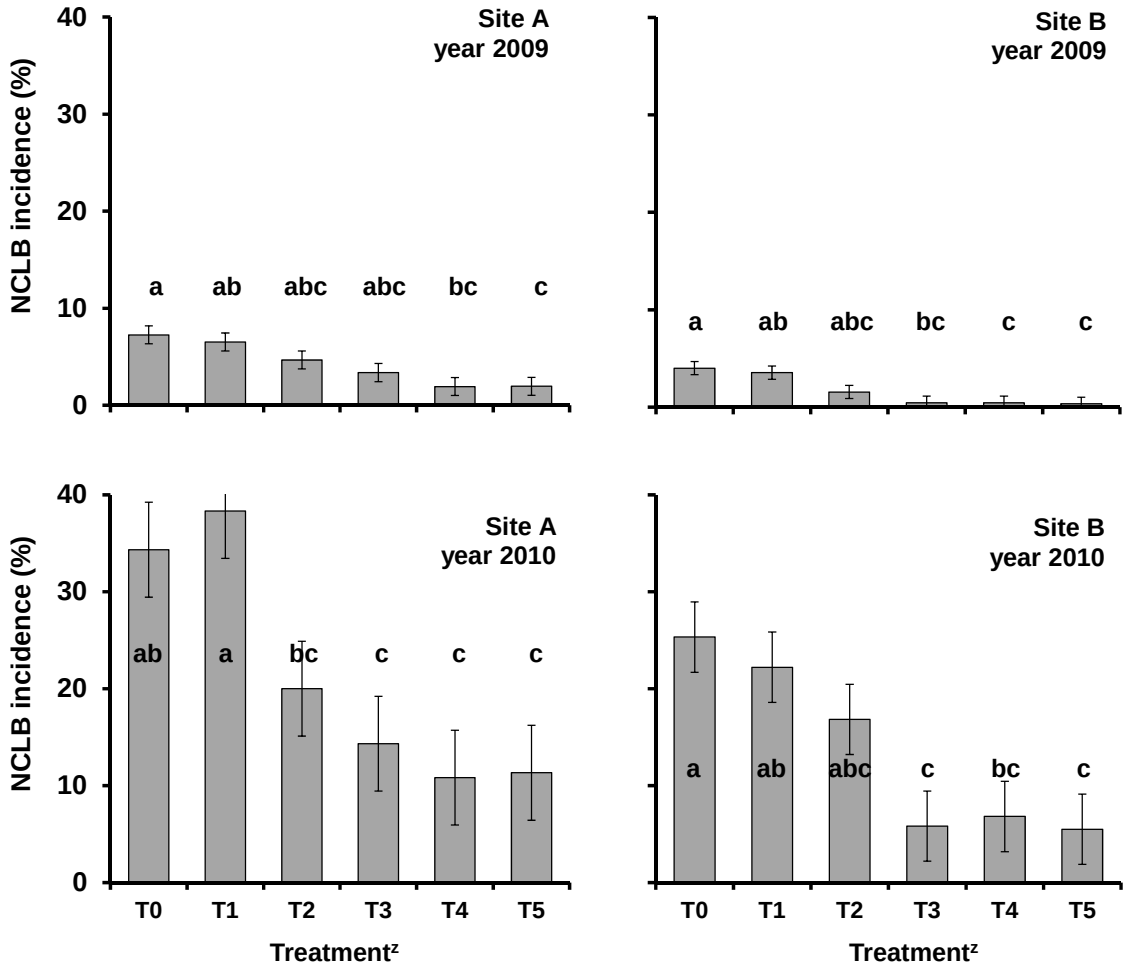
Means followed by different letters are significantly different (the level of significance is shown in the table). The reported values of the trial factor are based on 24 replications (6 treatment X 4 repetitions), while the values of the treatment factor are based on 16 replications (4 trials X 4 repetitions).

The ECB and fungal ear rot severity means reported are transformed (T; y'=arcsin$\sqrt{x}$*180/π) and not transformed (N) values. The fumonisin contamination means reported are transformed [T; y'= ln (x + 1)] and not transformed (N) values.

[Y] Treatment: T0, untreated control; T1, fungicide application at leaf development at 4 unfolded leaves (GS 14); T2, fungicide application at end of leaf development (GS 19); T3, fungicide application at middle of stem elongation with 5 detectable nodes (GS 35); T4, fungicide application at flowering with fully emerged stigmata (GS 65); T5, fungicide application at milk stage (GS 75).[Z] sem: standard error of mean

Figure 1.

Effect of the azoxystrobin + propiconazole application timing on Northern Corn Leaf Blight (NCLB) incidence[y], field experiments conducted in 2 sites in the 2009 - 2010 period.



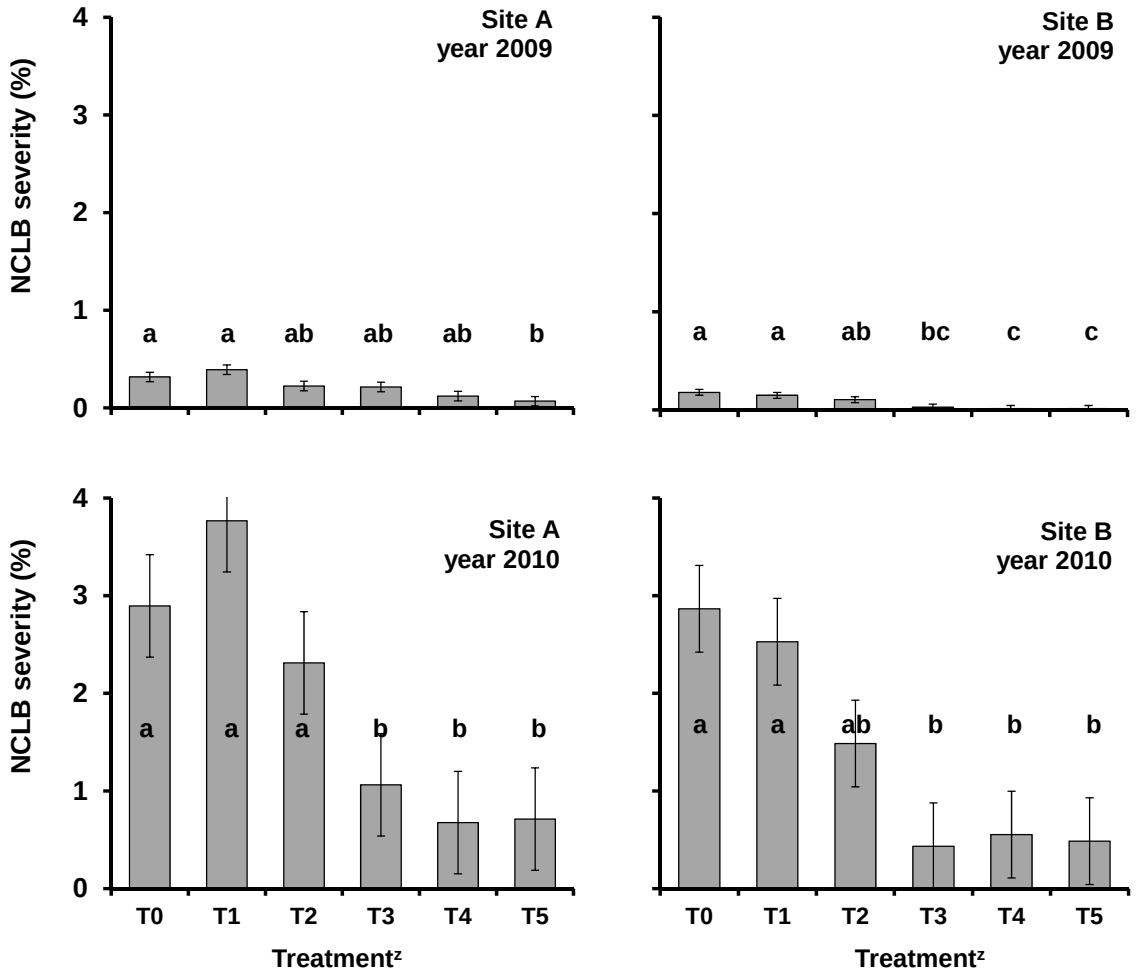
[y] NCLB incidence was calculated as the percentage of leaves with NCLB symptoms at the dough stages (GS 85), based on 4 replications of 75 leaves each.

[z] Treatment: T0, untreated control; T1, fungicide application at leaf development at 4 unfolded leaves (GS 14); T2, fungicide application at end of leaf development (GS 19); T3, fungicide application at middle of stem elongation with 5 detectable nodes (GS 35); T4, fungicide application at flowering with fully emerged stigmata (GS 65); T5, fungicide application at milk stage (GS 75).

The means followed by different letters are significantly different ($P < 0.05$) for each site and year combination. The reported values are based on 4 replications. The error bars indicate the standard error of means.

Figure 2.

Effect of the azoxystrobin + propiconazole application timing on Northern Corn Leaf Blight (NCLB) severity[y], field experiments conducted in 2 sites in the 2009 - 2010 period.



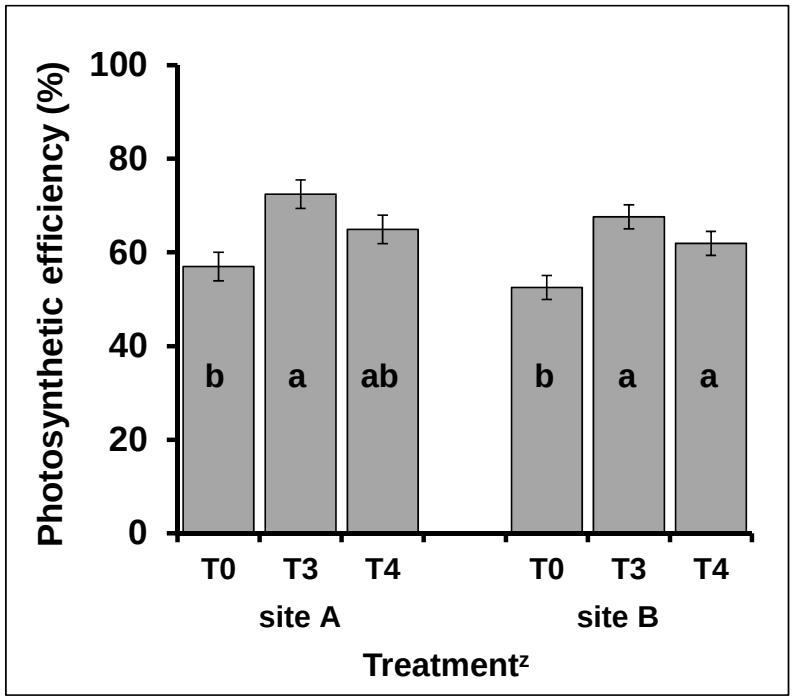
[y] NCLB severity was calculated as the mean percentage of leaf surfaces with symptoms of disease at the dough stages (GS 85), based on 4 replications of 75 leaves each.

[z] Treatment: T0, untreated control; T1, fungicide application at leaf development at 4 unfolded leaves (GS 14); T2, fungicide application at end of leaf development (GS 19); T3, fungicide application at middle of stem elongation with 5 detectable nodes (GS 35); T4, fungicide application at flowering with fully emerged stigmata (GS 65); T5, fungicide application at milk stage (GS 75).

The means followed by different letters are significantly different ($P < 0.05$) for each site and year combination. The reported values are based on 4 replications. The error bars indicate the standard error of means.

Figure 3.

Effect of the azoxystrobin + propiconazole application timing on photosynthetic efficiency[y], field experiments conducted in 2 sites in 2010.



[y] The photosynthetic efficiency is defined by the ratio between the difference in leaf nonstructural carbohydrates (LNSC) content at sunset and at sunrise and the LNSC at sunset.

[z] Treatment: T0, untreated control; T3, fungicide application at middle of stem elongation with 5 detectable nodes (GS 35); T4, fungicide application at flowering with fully emerged stigmata (GS 65).

The means followed by different letters are significantly different ($P < 0.05$) for each site. The reported values are based on 4 replications. The error bars indicate the standard error of means.