Effect of high planting density and foliar fungicide application on the grain maize and silage and methane yield

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Abstract

The research investigated ways to enhance maize yield in intensive maize cropping system by evaluating the effect of high planting densities combined with foliar fungicide treatments. The considered assessments were fungal leaf disease, biomass and grain yield and methane production through anaerobic fermentation. The experiment was conducted in the years 2012 and 2013. The treatments compared at each location were factorial combinations of two plant densities and three fungicide applications. A standard planting density (StD, 7.5 plants m^{-2} on a 0.75 m inter-row spacing) was compared with the high density (HiD, 10 plants m^{-2} on narrow 0.5 m inter-row spacing). Two fungicides, pyraclostrobin at 0.2 kg AI ha^{-1} and a mixture of pyraclostrobin and epoxiconazole at 0.2 and 0.075 kg AI ha^{-1} respectively, were applied at the tassel emergence stage and compared with an untreated treatment. The HiD system positively increased the silage maize yield (+16%), grain (+17%) and methane yield per hectare (+19%) in comparison to the StD. The fungicide application significantly restrained foliar disease symptoms only in 2012. Fungicide did not affect plant silage composition (protein, starch or fibre content) and methane yield, conversely it significantly increased grain yield for both planting density systems (+5%). The overall boost in yield obtained by combining both strategies in an intensive system, HiD combined with the fungicide, was +24% for methane and +21% for grain yield compared to StD without fungicide application. This work proved that an intensive high planting system with up to 10 plants m^{-2}, supported by leaf fungicide treatments, can lead to a real yield enhancement of both maize grain and silage.

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Introduction

Maize (*Zea mays* L.) is one of the most important crops cultivated throughout the world, because of its high productivity of both grain and whole plant biomass, which can be used in the food, feed and industrial sectors (Lee *et al*., 2007). Recently, there has been growing interest in the use of this crop as a renewable energy source (Seleiman *et al*., 2013). Although there are several ways of producing energy from maize biomass, the production of biogas, mainly composed of methane, from anaerobic fermentation is one the most common ways in some areas of the European Union (Herrmann and Rath, 2012). Maize biomass, along with livestock waste, is one of the main components used for this process, since it has the highest yield potential of all the crops cultivated in Central and South Europe (Amon *et al*., 2007) and it offers a suitable substrate for anaerobic fermentation (Negri *et al*., 2014).

The increasing competition in the use of maize for food, feed and energy purposes, considering both grain and silage, has led to the need to find new agronomic solutions able to face the increasing demand for this crop for different purposes (Seleiman *et al*., 2017). A possible way of increasing the crop and single plant yield potential is by applying agronomic solutions that are able to enhance the interception of photosynthetic active radiation (IPAR) (Maddonni *et al*., 2006). One way of achieving this goal is by optimizing the planting pattern in a new setup able to support higher plant population, allowing a more efficient light interception (Duvick, 2005; Ottman and Welch, 1989). Another strategy is by protecting the leaf health and prolonging the stay green effect, allowing a better and longer light interception capacity (Testa *et al*., 2015).

As far as the planting pattern is concerned, nowadays hybrids are conceived to better bear the higher stress that occurs when high planting density systems are applied (Tollenaar, 1989; Widdicombe and Thelen, 2002). However, even for these modern plants, the best yield response has been observed when the equidistance space between plants was maximized (Lauer, 1994). Therefore, the high plant population characterized by a narrow inter-row system leads to a greater distance from contiguous plants in the same row, and offers a better growing environment than the same high density sown in wide rows (Sangoi, 2000).

Moreover, a higher foliar disease pressure occurs more often under intensive planting when interplant distance is reduced (Adipala *et al*., 1995). In order to increase crop outputs through a higher planting density, minimizing problems linked to a more stressful condition, which reduce the single plant yield potential, the use of foliar fungicides can be crucial. This second strategy, which relies on single plant efficiency, consists on the application of a quinone outside inhibitor (QoI) fungicide (strobilurin), applied alone or combined with a demethylation inhibitor (DMI) (azole), in order to restrain fungal development on leaves (Blandino *et al*., 2012). The most common foliar disease for maize grown in North Italy is northern corn leaf blight (NCLB), which is mainly caused by *Exserohilum turcicum* (Bowen and Pedersen, 1988). It results in elliptical lesions, which generally develop on leaves after flowering, and leads to a premature leaf senescence (Munkvold and Gorman, 2006), a lower IPAR and, consequently, a lower plant yield potential. The magnitude of yield loss mainly depends on two factors: the severity of the disease and the plant growth stage at which the infection occurs (Perkins and Pedersen, 1987). In addition to disease control, QoI fungicides could lead to plant benefits other than disease control, such as improved environmental stress tolerance and longer maintenance of green leaf area during ripening. Since plant density and fungicide application intervene on different aspects of the yield potential, these crop techniques can be combined in intensive maize cropping systems. To the best of the authors' knowledge, no previous studies were conducted to evaluate the interaction between these two agronomical strategies. Therefore, the aim of this study was to evaluate the combination of high plant population and fungicide application on grain yield and whole plant yield for silage, in particular considering methane production by means of anaerobic fermentation.

Materials and methods

Experimental site and treatments

Three field trials were carried out in the 2012 and 2013 growing seasons in North West Italy. The experiments were carried out in Carignano in 2012 and in Buriasco and Vigone in 2013 in the same agricultural area in the Piedmont region in North West of Italy, characterized by a humid subtropical climate according to the Köppen climate classification (Peel, 2011). The main agronomic information of each trial is reported in Table 1.

The treatments compared at each location were factorial combinations of:

- Two plant densities: StD, a standard planting density (7.5 plants m^{-2}) sown at a 0.75 m wide inter-row spacing and an average distance of 0.18 m between two contiguous plants and HiD, an high planting density (10 plants m^{-2}) with a narrow inter-row spacing of 0.5 m and a distance between plants of 0.2 m in the same row.
- Three fungicide applications at maize tassel emergence (growth stage, GS 51; Bleiholder *et al*., 2001): i) untreated treatment; ii) PYR: the application of pyraclostrobin active ingredient (AI) (Methyl [2-({[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxy}methyl)phenyl]methoxycarbamate) at 0.2 kg AI ha^{-1} (Retengo new®, 1 l ha^{-1} of formulate, BASF, Cesano Maderno, Italy); iii) PYR + EPO, the application of a mixture of pyraclostrobin and epoxiconazole ((2RS,3SR)-1-[3-(2-chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl)propyl]-1H-1,2,4-triazole) at 0.2 and 0.075 kg AI ha^{-1}, respectively (Retengo Plus®, 1.5 l ha^{-1} of formulate; BASF, Cesano Maderno, Italy).

The treatments were assigned to experimental units using a split-plot design, with the planting density as the main-plot treatment and the fungicide application as the sub-plot treatment. Each treatment was replicated 4 times. The 10 m long sub-plot with a surface of 60 m^2, consisted of 8 rows and 12 rows for the StD and HiD planting systems, respectively. The plot alleys, orthogonal to the maize rows, were one meter wide.

Studies were carried out on the Pioneer P1547 commercial dent maize hybrid at Carignano in 2012 and at Buriasco in 2013, and on the Pioneer PR34G44 hybrid at Vigone in 2013, both of which belong to FAO maturity class 600 (130 days relative to maturity), with medium susceptibility to NCLB.

In all experimental fields, the previous crop was grain maize. To prepare the proper seedbed, sowing was carried out after an autumn 0.3 m deep ploughing, followed by disk harrowing. All the experimental fields received 250, 100 and 100 kg ha^{-1} of N, P_2O_5 and K_2O, respectively. All the potassium was applied before sowing, whereas 200 kg ha^{-1} of diammonium phoshate ere applied at sowing and the remaining nitrogen, through urea at 6 leaves stage (BBCH16). All the sites were irrigated twice during the growing season 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-metolaclor (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 plots were sprayed, at GS 75, with pyrethroid alpha-cypermetrin insecticide (Contest®, BASF, Cesano Maderno, Italy) at 0.2 kg AI ha^{-1}, in order to restrain the damage to the ears caused by the

Table 1. Main site and soil information concerning the 3 experiments.

Factor	Experiment Carignano	Buriasco	Vigone
Growing season	2012	2013	2013
N coordinates	44° 52' 53''	44° 51' 54''	44° 50' 19''
E coordinates	7° 37' 37''	7° 26' 21''	7° 28' 54''
Altitude (m) (a.s.l.)	242	264	264
Soil (USDA classification)	Aeric Fluvaquents	Typic Udifluvents	Typic Udifluvents
Sand (%)	38.5	43.8	39.8
Silt (%)	53.3	45.9	54.7
Clay (%)	8.2	10.3	5.5
Organic matter (%)	3.2	1.7	1.9
C/N	9.9	9.1	10.1
N (g kg^{-1})	1.9	1.1	1.1
Available P_2O_5 (ppm)	125	42	22
Exchangeable K_2O (ppm)	74	68	201
Cation exchange capacity (meq 100 g^{-1})	14.1	14.0	9.2
pH	7.3	6.3	8.0

European Corn Borer (*Ostrinia nubulalis* Hübner). All the fungicide and insecticide treatments were carried out using a self-propelled ground sprayer (Eurofalcon E140®, Finotto) with a hydraulically adjustable clearance. Flat-fan nozzles were used to spray a volume of 400 L ha^{-1}, at a pressure of 200 kPa. A fan was used to blow low-pressure air towards the crop, while the nozzles were spraying, to increase the penetration of the chemical product into the canopy. The operation speed was 10 km h^{-1}. The planting and harvest dates, as well as the fungicide treatments, are reported in Table 2 for each year and site.

Leaf fungal disease

Fifteen plants per plot were visually evaluated at flowering (GS 63), during the milk stage (GS 75) and dough stage (GS 85), in order to establish the incidence and severity of NCLB symptoms. Five leaves were considered for each plant: the ear leaf and the 2 leaves above and below the ear. Disease incidence was calculated as the percentage of leaves with symptoms (considering 75 leaves per plot), while severity was calculated as the average percentage of leaf surface with symptoms. An index from 1 to 7 was used, in which each numerical value corresponded to a percentage interval of foliar surfaces exhibiting visible symptoms, according to the following scale: 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 leaf surface showing symptoms and each score was replaced with the mid-point of the interval (Blandino *et al*., 2012).

Biomass and methane yield

This assessment was only performed at Carignano in 2012 and at Vigone in 2013. Ten whole plants were collected manually from each plot at the dough stage (GS 85). The plant samples were weighed to establish the biomass yield and then passed through a field chopper. About 1 kg of chopped subsample was weighed before and after being dried at 105°C for 48 h to establish the dry matter (DM) content. The 4 field replicates of fresh matter belonging to the same treatment were merged and analysed for the volatile solid content (VS), protein content, starch, simple carbohydrate and ash content, neutral detergent fibre (NDF), acid detergent fibre and acid detergent lignin through a near infrared instrument (NIR Systems scanning 5000 monochromator, Foss UK Ltd., Warrington, UK) (Lovett *et al*., 2005). Prior to scanning, each samples was dried a 60°C for 48 h and the homogenized material was then passed through a 1 mm screen using a Cyclotec mill (Perstorp Analytical Ltd., Bristol, UK). Spectra were obtained for each sample at 2 nm intervals over the 1100-2500 nm wavelength range.

The specific methane yield per ton of VS was measured through the biochemical methane potential (BMP) method (Owen *et al*., 1979), according to the procedure described in the UNI EN ISO 11734:2004 standard, and expressed as methane production per hectare. These samples were stored in a refrigerator at a constant temperature (4°C) under vacuum for a maximum of 2 weeks before analysis.

Grain yield, ear and kernel traits

The ears were collected by hand from each plot at the end of maturity, at grain moisture content of between 21-29%. The sampling area was 4.5 m^2 and 3 m^2 on plots where the inter-row spacing was respectively wide and narrow (2 rows X 3 m long). The ear length, excluding the tip-back without kernels, was measured on a sub-sample of 15 randomly chosen ears. In order to establish the grain yield, all the ears harvested in 2012 were passed through an electric sheller. The obtained kernels were then weighed and the grain moisture was recorded by means of a Dickey-John GAC2000 grain analysis meter (Dickey-John Corp. Auburn, IL, USA). In 2013, the grain yield was obtained by harvesting the whole plot using a plot scale combine harvester. Further evaluations were performed on kernels dried for 72 h at 60°C: test weight (TW), measured by means of a Dickey-John GAC2000 grain analysis meter, and thousand kernel weight (TKW), calculated by counting and weighing 200 randomly selected kernels. Two analytical replicates were carried out for each grain moisture, TW and TKW assessment.

Statistical analysis

The normal distribution and homogeneity of variances were verified by performing the Kolmogorov–Smirnov normality and Levene test. The analysis of variance (ANOVA) was run, using a completely randomized split plot design in order to analyse the effect of planting density, fungicide application and their interaction, by considering the experiment (combination of site and year) as a random factor. When necessary, post-hoc multiple comparison tests were performed, according to the Ryan-Einot-Gabriel-Welsh F test, on the fungicide treatment factors.

The effect of planting density and fungicide application on the NCLB symptoms was verified by keeping the different trials separate, since a dissimilar disease pressure was observed between sites and growing seasons. The incidence and severity values of NCLB were previously transformed using $y' = \arcsin\sqrt{x} * 180/\pi$, as percentage data derived from counting. SPSS Version 24 for Windows was used for the statistical analysis.

Results

The two growing seasons differed from each other in terms of rainfall and temperature, mainly during spring time (Table 3). The abundant rainfall that occurred in 2013 from April to May caused a delayed planting and slowed down the crop vegetative growth during the first stages, with a consequent late ripening period. On the other hand, the 2012 growing season was warmer and drier than 2013 from June to August.

The foliar disease attacks in the considered growing seasons were moderate. In 2012, no NCLB symptoms were observed at the flowering stage (GS 63) whereas during the milk (GS 75) and dough (GS 85) stages the disease was visible on leaves (Table 4). The incidence and severity of leaves showing symptoms at the dough stage (GS 85) was 28% and 1.1%, respectively. In the 2013 growing season, NLCB symptoms on leaves were negligible on all

Table 2. Main agronomical information concerning the 3 experiments.

Factor	Experiment		
	Carignano	Buriasco	Vigone
Hybrid	P1547	P1547	PR34G44
Sowing	27 Mar	13 May	13 May
Fungicide treatment	2 Jul	23 Jul	23 Jul
Insecticide treatment	15 Jul	6 Aug	6 Aug
Silage biomass harvest	22 Aug	NP	12 Sept
Grain harvest	8 Oct	22 Oct	22 Oct

NP, not performed.

Table 3. Total monthly rainfall, rainy days, average temperature and growing degree days, from April to October, in all the tested locations.

Location (year)	Carignano (2012)			Vigone/Buriasco (2013)*		
Month	Rainfall (mm)	Rainy days (n)	GDD° (°C d^{-1})	Rainfall (mm)	Rainy days (n)	GDD (°C d^{-1})
April	140	19	112	206	17	124
May	144	14	245	231	21	182
June	12	8	373	42	9	326
July	37	10	408	93	8	428
August	31	9	422	25	9	405
September	53	13	265	11	7	297
October	63	15	162	85	15	148
April-October	480	88	1987	694	86	1911

*Vigone and Buriasco refer to the same meteorological station; °GDD, accumulated growing degree days per month using a 10°C base. Source: Rete Agrometeorologica del Piemonte - Regione Piemonte - Assessorato Agricoltura - Settore Fitosanitario, sezione di Agrometeorologia.

Table 4. Effect of planting density and fungicide treatments on northern corn leaf blight (NCLB) incidence and severity in Carignano location in the 2012 growing season.

Factor	Source of variation	NCLB milk stage (GS 75)		NCLB dough stage (GS 85)	
		Incidence (%)	Severity (%)	Incidence (%)	Severity (%)
Plant density°	StD	2.11^a	0.04^a	25.7^a	0.94^b
	HiD	1.33^a	0.02^a	29.8^a	1.34^a
	P (F)	ns	ns	ns	*
Fungicide treatment#	Untreated	2.67^a	0.06^a	33.7^b	1.73^c
	pyr	1.17^a	0.02^a	28.8^b	1.11^b
	pyr + epo	1.33^a	0.02^a	20.7^a	0.58^a
	P (*F*)	ns	ns	**	***
Plant density X fungicide	P (*F*)	ns	ns	ns	ns

Means followed by different letters are significantly different (the level of significance is shown in the table: *P<0.05, **P<0.01, ***P<0.001, ns not significant). °The planting density factor values are based on 12 replicates (3 treatments × 4 repetitions). StD, standard planting density, 7.5 plant m^{-2} on 0.75 m inter-row spacing; HiD, high planting density, 10 plants m^{-2} at a 0.5 m inter-row spacing; #The fungicide treatment values are based on 8 replicates (2 crop densities × 4 repetitions). The treatment theses are: untreated control; application of pyraclostrobin at GS 51; application of pyraclostrobin and epoxiconazole mixture at GS 51.

locations and during all three phenological growth stages evaluated. In 2012 growing season, the severity of NCLB at dough stage was 44% greater for HiD than for StD. The fungicide application treatments significantly restrained NCLB development on the leaves evaluated during GS 85. At this stage, the pyraclostrobin and epoxiconazole mixture resulted in a significant reduction of the disease symptoms for both incidence (–39%) and severity (–67%). On the other hand, the application of the pyraclostrobin AI alone only significantly reduced the severity of the disease (–36%), but not the NCLB incidence.

The effect of planting density and fungicide treatments on the silage yield, and methane yield is summarized in Table 5. The increase in plant population significantly increased the silage yield if compared to the standard density (+16%). Conversely, no significant difference on plant biomass was reported for fungicide application. Both plant density and fungicide application did not affect considerably the biomass DM, VS, protein, starch, soluble carbohydrate and ash content and the fibre composition (Table 6). The methane yield per ton of VS measured through the BMP method was on average 310 Nm^3 t^{-1} VS for the StD and 326 Nm^3 t^{-1} VS for the HiD system.

Table 5. Effect of plant density and fungicide treatments on the silage yield, dry matter (DM) and methane yield per hectare for the field experiments carried out in Carignano (2012) and Vigone (2013).

Factor	Source of variation	Silage yield t ha^{-1} DM	DM yield %	Methane Nm^3 ha^{-1}
Plant density°	StD	28.1^b	34.9^a	7808^b
	HiD	32.6^a	34.9^a	9334^a
	P (F)	***	ns	***
Fungicide treatment#	Untreated	29.8^a	34.2^a	8679^a
	pyr	30.4^a	36.6^a	8326^a
	pyr + epo	30.8^a	34.0^a	8707^a
	P (*F*)	ns	ns	ns
Plant density X fungicide	P (*F*)	ns	ns	ns

Means followed by different letters are significantly different (the level of significance is shown in the table: *P<0.05, **P<0.01, ***P<0.001, ns not significant). °The planting density factor values are based on 24 replicates (2 locations × 3 treatments × 4 repetitions) StD, standard planting density, 7.5 plant m–2 at a 0.75 m inter-row spacing; HiD, high planting density, 10 plants m^{-2} at a 0.5 m inter-row spacing; #The fungicide treatment values are based on 16 replicates (2 locations × 2 crop densities × 4 repetitions). The treatment theses are: untreated control; (pyr) application of pyraclostrobin at GS 51; (pyr + epo) application of pyraclostrobin and epoxiconazole mixture at GS 51.

As far as the fungicide application is concerned, the methane yield per ton of VS was on average 319, 321 and 313 Nm^3 t^{-1} VS for the untreated control, the pyraclostrobin alone and the pyraclostrobin and epoxiconazole treatment, respectively.

The methane yield per hectare was significantly enhanced by means of the higher plant population (Table 5) with an average increase of 19%. The effect of fungicide application and the interaction between plant density and fungicide application were never significant on plant biomass parameters.

The higher number of plants per unit surface (HiD system), obtained from the narrow inter-row spacing, significantly influenced the grain yield, grain moisture, TKW and ear length (Table 7). The HiD system in fact, compared to the StD one, on average increased grain yield by 16.8%. Furthermore, the kernel moisture at harvest was 2.1% higher in the HiD system, whereas the TKW was 3.8% lower. No differences were observed for the TW values, whereas the ear length, a parameter correlated with the single plant yield potential, was significantly reduced (–9%) in the HiD system compared to StD. The application of the pyraclostrobin and epoxiconazole mixture significantly increased grain yield (+5.1%) and moisture (from 29.3% to 30.2%), compared to the untreated control, and the TKW also showed an increasing trend (+2.9%). The interaction between the plant density and fungicide treatment was never significant. The overall yield boost obtained from combining both strategies in an innovative system, characterized by the HiD treated with the fungicide, was +21% for methane and +24% for grain yield (Figure 1).

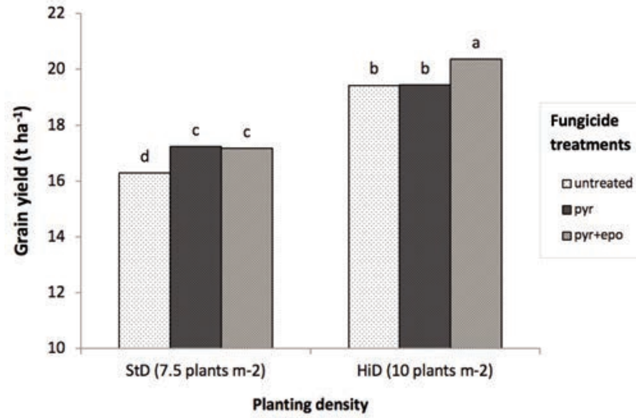

Figure 1. Average grain yield for the agronomical strategies obtained from the factorial interaction of planting density and fungicide treatment. The agronomical strategy values are based on 12 replicates (3 experiments × 4 repetitions). Bars with different letters are significantly different (P<0.05). The interaction between the compared treatments and the experiment was not significant (P>0.05). Planting density: StD, standard planting density, 7.5 plant m^{-2} at a 0.75 m inter-row spacing. HiD, high planting density, 10 plants m^{-2} at 0.5 m inter-row. Fungicide treatment: untreated control; (pyr) application of pyraclostrobin at GS 51; (pyr + epo) application of pyraclostrobin and epoxiconazole mixture at GS 51.

Table 6. Maize silage composition of crop with different plant density and fungicide application for the field experiment carried out in Carignano (2012) and Vigone (2013).

Factor	Source of variation	VS (%)	Ash (%)	PC (%)	Starch (%)	SC (%)	NDF (%)	ADF (%)	ADL (%)
Plant density°	StD	95.9	1.2	7.9	27.2	1.2	44.2	28.0	3.6
	HiD	97.4	1.3	7.8	26.8	1.3	45.4	28.7	3.7
Fungicide treatment#	Untreated	97.1	1.2	7.8	25.6	1.2	46.5	29.6	3.7
	pyr	96.2	1.1	7.8	27.6	1.1	44.0	27.9	3.6
	pyr + epo	96.7	1.4	7.8	27.8	1.4	43.9	27.5	3.5

VS, volatile solid content; PC, protein content; SC, simple carbohydrate content; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin. °The planting density factor values are based on 24 replicates (2 locations × 3 treatments × 4 repetitions) StD, standard planting density, 7.5 plant m^{-2} at a 0.75 m inter-row spacing; HiD, high planting density, 10 plants m^{-2} at a 0.5 m inter-row spacing; #The fungicide treatment values are based on 16 replicates (2 locations × 2 crop densities × 4 repetitions). The treatment theses are: untreated control; (pyr) application of pyraclostrobin at GS 51; (pyr + epo) application of pyraclostrobin and epoxiconazole mixture at GS 51.

Table 7. Effects of planting density and fungicide treatments on the grain yield, kernel moisture, thousand kernel weight (TKW), test weight (TW) and ear length for the field experiments carried out in Carignano (2012), Vigone and Buriasco (2013).

Factor	Source of variation	Grain yield ($t\ ha^{-1}$)	Moisture (%)	TKW (g)	TW ($kg\ hL^{-1}$)	Ear length (cm)
Plant density°	StD	16.9^{b}	29.4^{b}	412^{a}	80.3^{a}	19.0^{a}
	HiD	19.8^{a}	30.0^{a}	396^{b}	80.3^{a}	17.3^{b}
	P (*F*)	***	*	**	ns	***
Fungicide treatment#	Untreated	17.9^{b}	29.3^{b}	397^{a}	80.4^{a}	18.0^{a}
	pyr	18.3^{ab}	29.7^{ab}	407^{a}	80.1^{a}	18.2^{a}
	pyr + epo	18.8^{a}	30.2^{a}	408^{a}	80.3^{a}	18.2^{a}
	P (*F*)	*	*	ns	ns	ns
Plant density X fungicide	P (*F*)	ns	ns	ns	ns	ns

Means followed by different letters are significantly different (the level of significance is shown in the table: *P<0.05, **P<0.01, ***P<0.001, ns not significant). °The planting density factor values are based on 36 replicates (3 locations × 3 treatments × 4 repetitions) StD, standard planting density, 7.5 plant m^{-2} at a 0.75 m inter-row spacing; HiD, high planting density, 10 plants m^{-2} at a 0.5 m inter-row spacing; #The fungicide treatment values are based on 24 replicates (3 locations × 2 crop densities × 4 repetitions). The treatment theses are: untreated control; (pyr) application of pyraclostrobin at GS 51; (pyr + epo) application of pyraclostrobin and epoxiconazole mixture at GS 51.

Discussion and conclusions

The study, through a comparison of different sites and growing seasons, indicated that a higher planting density can positively influence silage and grain maize yields, while the application of foliar fungicide resulted in higher advantage for grain yield.

As far as the silage production is concerned, a planting density higher than 10 plants m^{-2} for full season hybrids improves the whole plant biomass yield, which is in agreement with Cox and Cherney (2001). Moreover, since the methane yield per VS in the HiD planting system was not reduced compared to the StD one, the greater biomass caused a clear increase of methane yield per land area. Conversely, the fungicide application did not show any yield advantage, in terms of silage biomass, or a clear positive methane yield enhancement trend. The absence of silage yield benefits could be linked to the lack of intense fungal attacks on leaves in the considered growing seasons. Haerr *et al*. (2015) have reported a constant biomass yield and an increased biomass quality, in terms of feed dry matter on maize treated with pyraclostrobin and metconazole at different phenological stages. Blonde and Esker (2008) have reported an enhancement of forage quality, due to a pyraclostrobin treatment applied at the tassel emergence stage (GS 51), in terms of DM yield and lower NDF, which resulted in a higher milk energy yield. A further indirect advantage of fungicide application to maize for silage is that the prolonged stay green can lengthen the harvesting period during the dough stage. Overall, these better conditions could lead to a potentially further higher biomass energy content and methane yield.

The grain yield was increased by means of both agronomical strategies considered. The increase of plant population resulted in the highest and more clear grain yield enhancement. However, even with an appropriate inter-row spacing, on high densities systems, the single plant is forced to face a more stressful condition, which leads to an inevitable detriment of the single plant yield potential. In fact, by increasing the plant density from 7.5 to 10 plants per square meter (+33%), the obtained average grain yield gain did not result to be proportional (+16.8%). This result is in agreement with previous data obtained in North Italy in which StD and HiD were compared in several production situations (Testa *et al*., 2016). The lower single plant yield potential that resulted from the higher plant density is mainly caused by a lower kernel weight (Haegele *et al*., 2014) and higher ear barrenness, as testified also by the shorter ears. Moreover, in the growing season with the occurrence of disease, the plots sown at high densities resulted in a slightly higher NCLB development on the leaves. This greater development was probably due to the higher stressful environment the plants were forced to cope with, which led to a lower capability of competing against fungal development. A similar response to different planting densities on NCLB was also reported by Adipala *et al*. (1995); therefore, a growth of foliar disease attack linked to the increase of the plant density is plausible in growing seasons that promote fungal development. Although the foliar disease pressure was low, particularly in 2013 growing season, the DMI and QoI fungicide treatments can mitigate the stressful condition that occurs in these intensive planting systems, and can provide an additional tool that enhances plant recovery and preserves a good grain yield potential, mainly by increasing the TKW (Blandino *et al*., 2012). Unlike the biomass yield at dough stage, the fungicide application, acting on IPAR in a longer maturation period, could have led to grain yield advantages. In fact, the yield enhancement of +5%, obtained by means of the fungicide sprayings during the tassel emergence stage, has confirmed the data reported in previous experiments, in which similar compounds were tested on maize sown at a standard planting density (Munkvold *et al*., 2001; Paul *et al*., 2011; Testa *et al*., 2015). It has been reported that both active ingredients play roles in stimulating photosynthesis (Gooding *et al*., 2000; Petit *et al*., 2012). QoI-containing fungicides have in general been shown to provide greater physiological benefits and to increase grain yield through enhanced plant performance, even during the absence of disease in maize (Nelson and Meinhardt, 2011) or in other crops (Bertelsen *et al*., 2001; Kato *et al*., 2011). The fungicide physiological enhancement effect allows a better translocation of the metabolic resources (starch) from the source organs (leaves) to the sink (ear), as it prevents their allocation from actively defending the plant against the pathogen (Petit *et al*., 2012; Testa *et al*., 2015). Conversely, the addition of epoxiconazole AI to the QoI fungicide has confirmed, as other DMI fungicides, to have a greater ability of restraining NCLB development (Bowen and Pedersen, 1988). Therefore, it is expected that with high disease pressure the use of DMI and QoI fungicide mixture could slow down more clearly the leaf senescence process, leading to higher single plant yield improvement.

In conclusion, the application of these two crop strategies could enhance maize yield, in terms of grain and biomass. Among the considered techniques, increasing the planting density to 10 plants m^{-2}, by maintaining an appropriate plant equidistance, has led to the best yield enhancement. The fungicides applications resulted in less pronounced yield benefits. On the other hand this practices could minimize the occurrence of earlier leaf senescence and preserve a higher yield potential of the single plant, by maintaining a consistent kernel weight at harvest. In these conditions, fungicide can come to the aid of high population systems, by offering the crop protection and the physiological plant benefits, and, ultimately, by minimizing the plant stress risks with these intensive growing conditions. Since these two agronomical strategies gravitate around two different aspects of the final yield, they can be successfully applied together in intensive maize cropping system to obtain a combined effect that allows greater benefits concerning grain, silage and methane yields.

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