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Organic *vs* conventional farming: Differences in infection by mycotoxin-producing fungi on maize and wheat in Northern and Central Italy

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ABSTRACT

This study aimed to monitor the main toxigenic fungi in neighbouring organic and conventional maize and wheat fields in Italy in 2010 and 2011. The *Fusarium* species mainly isolated were: *Fusarium poae*, sometimes predominant on *Fusarium graminearum* in wheat, and *Fusarium verticillioides* competing with *Fusarium proliferatum* and *Fusarium subglutinans* in maize. The incidence of *Fusarium* spp. was similar for both conventional (6%) and organic (4%) wheat, but it was influenced by weather conditions. 2010 was the most favourable for *Fusarium* species, with 10 times the incidence of 2011. *Fusarium* infection was significantly different between farming systems in maize (20% *vs* 35% in conventional and organic, respectively), while in 2010 the incidence was significantly higher than in 2011 (43% *vs* 25%). *Aspergillus* and *Penicillium* incidence was not linked to the farming system but to weather conditions, with moderately higher incidence in 2010.

1. Introduction

Organic farming, defined in Europe by the Commission Regulation (EC) No. 1991/2006 (a), amending Regulation (EEC) 2092/91, has significantly increased worldwide in the last two decades. Italy is the second largest area, after Spain, with 1.1 million ha of organic area, of European Countries (EC, 2013). Organic cereals are the second main aggregated crops cultivated in Italy, covering about 17% of the total organic area, with soft and durum wheat, and maize, accounting for 55% of the total organic cereals cultivated (SINAB, 2012).

It is estimated that 25% of the world's food production, including many basic foods, is affected by mycotoxin-producing fungi, with cereals (CAST, 2003), especially maize and wheat, contaminated at the highest levels. A review on mycotoxin occurrence between 2010 and 2013 on different cereals and related foodstuff showed that maize and wheat are, respectively, the first and the second most contaminated crops worldwide (Pereira et al., 2014).

The main mycotoxin-producing fungi affecting wheat and maize belong to the *Fusarium*, *Aspergillus* and *Penicillium* genera. In particular, *Fusarium* Head Blight (FHB) of wheat is caused by a complex of species responsible mainly for the accumulation in the kernels of trichothecenes, a family of potent mycotoxins causing inhibition of protein synthesis, and zearalenone (ZEA), an estrogenic compound (Desjardins, 2006). *Fusarium graminearum* is the main species producing deoxynivalenol (DON), the most common contaminant among the trichothecenes, and causing FHB of wheat and red-ear rot in maize (Logrieco et al., 2003). Moreover, the predominant occurring species can vary in different geographical areas and years, according to environmental conditions and agronomic practices, and each species can have its own mycotoxin profile (Logrieco et al., 2003). *Fusarium* ear rot of maize, one of the main diseases of this crop worldwide, is also caused by a complex of species, *Fusarium verticillioides*, *Fusarium proliferatum*, *Fusarium subglutinans* and the recently described *Fusarium temperatum* (syn. *F. subglutinans* group1, Scauflaire et al., 2011) being associated with the so called pink-ear rot (Logrieco et al., 2003). Among these species, *F. verticillioides* and *F. proliferatum* are the main species responsible for the production of fumonisins on kernels (Desjardins, 2006).

Aspergillus species belonging to section *Flavi* are known to produce aflatoxins (AFs), and are frequently reported worldwide to

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occur in maize kernels, especially in the tropical areas (Fandialan and Ilag, 2003; Kana et al., 2013). Conversely, few reports have shown contamination of wheat by AFs and at levels lower than 15 μg/kg (Biomin, 2013; Riba et al., 2010). In Italy, the occurrence of these mycotoxins on maize is an emerging problem and it is associated with seasons causing high water stress to maize plants (Piva et al., 2006). Finally, in agreement with Pitt et al. (2000), the main species associated with the accumulation of ochratoxin A (OTA) in cereal grains worldwide is *P. verrucosum*. In Italy this species has been rarely reported and OTA contamination of wheat can be considered a minor problem in the Italian environment (Logrieco and Moretti, 2008).

Several studies have been focused on the influence of farming systems on mycotoxin contamination in cereals. These reports have shown contrasting data on the level of *Fusarium* mycotoxin accumulation in organic *versus* conventional farming (reviewed in Köpke et al., 2007). In a Norwegian study, *Fusarium* species were isolated from oat, barley and wheat harvested in 2002–2004. Organic cereals were less infected by *Fusarium* and with a lower content of trichothecenes than conventional ones; moreover *Fusarium avenaceum*, *F. graminearum* and *Fusarium poae* were the predominant species (Bernhoft et al., 2010). The lack of crop rotation and the use of mineral fertilisers and pesticides, which are agricultural practices characterizing conventional farming *versus* organic, seem to be the most relevant reasons that cause the differences between the two growing systems (Bernhoft et al., 2012).

Regarding Italian cereals, as far as we are aware, there are no studies which focus on the comparison of fungal incidence in different farming systems. Infantino et al. (2012) described the *Fusarium* community associated with FHB in wheat harvested in organic farming located in different geographical areas in a three-year period (2004–2006). The study showed a low *Fusarium* incidence, *F. poae* being the most occurring species in all the three years. With respect to maize, there is a complete lack of data. In Europe, only one study was carried out in Spain by Ariño et al. (2007) on the fungal occurrence in maize harvested in 2001–2003, showing that total fungal contamination was higher in organic than in conventional maize, but *Fusarium* species predominated in the latter.

Due to this scarce information, knowledge regarding the occurrence of toxigenic fungi in both maize and wheat cultivated in organic farming would be welcome.

Therefore, the aims of this study were: i) to monitor the fungal population, in particular the main mycotoxin producing fungi, associated with organic maize and wheat collected from farms located in northern and central Italy; ii) to compare the incidence of mycotoxin producing fungi on maize and wheat cultivated following conventional and organic farming.

2. Materials and methods

2.1. Wheat and maize sample collection

Wheat and maize samples were collected in 2010 and 2011 from farms located in northern and central Italy. For each crop, neighbouring fields of conventional and organic farming were chosen, in order to reduce the variables influencing fungi associated with kernels.

A total of 101 wheat samples were collected in 2010 from 91 farms: 85 samples were cultivated as organic wheat (72 of soft wheat and 13 of durum wheat) and 16 were cultivated as conventional wheat (15 of soft wheat and 1 of durum wheat). In 2011, a total of 138 wheat samples were collected from 101 farms: 121 samples were cultivated as organic wheat (110 of soft wheat and 11 of durum wheat) and 17 were cultivated as conventional wheat (13 of soft wheat and 4 of durum wheat). For maize, 30 samples were collected in 2010 from 27 farms: 24 samples were cultivated as organic maize and 6 were cultivated as conventional maize. In 2011, 39 samples were collected from 33 farms, with 35 samples from organic cultivation and 4 from conventional maize fields. For each sample, all the farmers were asked to fill in a specific form which included relevant cropping system information: geographical coordinates, wheat variety or maize hybrid, soil texture, previous crop, debris management, tillage and other agronomic operations, sowing period and investment, mineral nutrition, weed control, flowering period, biotic and abiotic crop injuries, chemical control of pest (*Ostrinia nubilalis*, the European Corn Borer-ECB) and/or disease (FHB), harvesting period and moisture of kernels at harvesting.

Sampling was performed following the protocol described by the Commission Regulation (EC) N° 401/2006 (b); incremental samples of 100 g each were collected *in continuum* during harvest combine discharge to obtain a final sample of 10 kg. The samples were sent to the laboratory for mycological analysis; subsamples of 30 g were prepared and immediately processed.

2.2. Fungal isolation and morphological characterization

Fifty kernels were randomly selected from each subsample and surface sterilized by washing in ethyl alcohol (70%) for 10 min and with NaCl (1%) for 2 min followed by rinsing twice with sterile double distilled water. The kernels were then dried on sterile absorbent paper. Sterilized kernels were plated in 90 mm Ø Petri dishes filled with DCPA (Dichloran Chlorampenicol Peptone Agar) (Andrews and Pitt, 1986; modified using 20× less quantity of chloramphenicol) and incubated at room temperature for 5 days under ambient light.

Based on their phenotypic characteristics, colonies identified at genus level as *Aspergillus*, *Fusarium* and *Penicillium* were selected and single-spored (5 fold-serial dilution in peptone:water 1:100). *Aspergillus* and *Penicillium* single spores were transferred onto Czapek Agar (CZ) and, after 7 days, colonies were identified at section level for *Aspergillus* cultures and genus level for *Penicillium*, based on their morphology (Raper and Fennell, 1965; Pitt, 1979). Isolates belonging to the *Fusarium* genus, were identified at species level, according to Leslie and Summerell (2006), by using 3 media: Potato Dextrose Agar (PDA) (HIMEDIA, Mumbai, India), Carnation Leaf Agar (CLA) (Fisher et al., 1982) and Spezieller Nährstoffarmer Agar (SNA) (Nirenberg, 1976). The culture observations were performed on a Nikon Eclipse E50i microscope (Nikon, Japan; 600 X).

2.3. Molecular identification of Fusarium spp.

2.3.1. Fungal isolates and inoculum preparation

In order to confirm the morphological characterization results, a qualitative PCR analysis was run on randomly selected samples (10% of total isolates), covering all the morphologically identified species, recovered from both wheat and maize samples. Reference strains, stored at the Institute of Entomology and Plant Pathology-UCSC, Piacenza (MPVP) and the Institute of Sciences of Food Production-CNR, Bari (ITEM fungal collection, http://server.ispa.cnr.it/ITEM/Collection), were used as positive control for each species.

The strains were grown on PDA at 25 °C for 7 d in the dark. At the end of incubation, 10 mL of sterile distilled water was added to each plate and it was gently scraped to collect fungal conidia. The suspension was adjusted to 10^6 conidia/mL and 100 μL were inoculated in 100 mL Malt Extract Agar (MEA) (Pitt, 1979) liquid medium. The static cultures were incubated for 14 days at 25 °C in the dark, then freeze-dried overnight and stored at 4 °C. Two biological replicates were performed for each sample.

2.3.2. DNA isolation and PCR-identification

Total DNA was purified from lyophilized mycelium with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol and stored at −20 °C. The amount and quality of total DNA were estimated by Bio Photometer (Eppendorf, Hamburg, Germany). Species-specific primers used for isolate identity confirmation by PCR analyses were found in the literature. In particular, for the identification of *F. graminearum*, *F. poae, Fusarium langsethiae*, the specific primers used were based on the EF-1α gene sequences (Nicolaisen et al., 2009); for *F. verticillioides* and *F. proliferatum* primers were designed on ITS-region gene sequences (Visentin et al., 2009); *F. subglutinans* isolates were confirmed by using specific primers designed on the calmodulin gene by Mulè et al. (2004). Since *F. poae* and *F. langsethiae* are difficult to distinguish morphologically, we used both *F. poae* and *F. langsethiae* primer pairs for strains attributed to both species based on microscopic observations.

PCR amplifications were performed using a Mastercycler personal (Eppendorf) in the following conditions: an initial step at 95 °C for 3 min followed by 35 cycles at 95 °C for 40 s, 60 °C (*F. graminearum*, *F. poae*, *F. langsethiae* primers) or 62 °C (*F. verticillioides*, *F. proliferatum*, *F. subglutinans* primers) for 40 s and 72 °C for 40 s and finally 72 °C for 10 min. Each reaction mix contained 20 ng of cDNA, 0.45 μL of each primer (10 μM), 0.2 μL of Taq DNA polymerase (5 U/μL), 1.5 μL of 10× PCR buffer, 0.15 μL of $MgCl_2$ (50 mM), 0.3 μL of dNTPs (10 mM) and sterile nuclease free water up to a total volume of 15 μL. PCR results were visualized on electrophoretic gel.

2.4. Meteorological data

Daily meteorological data regarding air temperature (C°), relative humidity (RH, %) and rain (mm) were registered by the agrometeorological network in Emilia-Romagna, Italy. The period considered was 1st March to 30th September in order to include all the relevant growth period both for wheat and maize. The region is virtually covered by a grid of squares, each of them 5 km^2 wide, and meteorological data are estimated for each square based on all data sources available (meteorological stations and radar located no more than 10 km far from the field considered) (Bottarelli and Zinoni, 2002). Two representative sites both for wheat, Castelfranco Emilia (MO) and Medicina (BO), and maize, Mirandola (MO) and Copparo (FE), were chosen to show the data.

2.5. Statistical analyses

All statistical analyses were performed by using SPSS v.19.0 (SPSS Italia, Bologna, Italy). One-way analysis of variance (ANOVA) was carried out and the Tuckey test was applied to detect significant differences between means. ANOVA was performed on a selection of data, chosen with the following criteria. All the samples belonging to the "conventional" cropping system were associated with corresponding "organic" samples cultivated in the same location or in proximity, in order to exclude the influence of variables other than the cropping system (conventional/organic); therefore, the same number of organic and conventional fields was considered for this data analysis.

3. Results

3.1. Cropping system information

3.1.1. Organic wheat

Two hundred and six samples of organic wheat including durum (24) and soft (182) were collected in the geographical area situated between 43°16′00″–43°59′00″N, 11°59′00″–13°03′39″ E; and 40°02′10″–45°64′90″N, 10°00′00″–16°72′90″E respectively (Fig. 1). Six durum wheat varieties (San Carlo the most used) and 22 soft wheat varieties (most used: Artico, Blasco, Bolero and Bologna; only varieties with a sample number ≥5 were cited) were analysed for the organic farming system. The varieties of soft wheat belonged to all the 4 categories of the Synthetic Quality Index (ISQ) (Borasio, 1997): FB (for biscuits), FF (improved), FP (ordinary bread making), and FPS (superior red making quality), FPS being the predominant category (≈76%). Almost all the farms practiced crop rotation. Previous crops were classified as legumes, cereals, maize, industrial crops, forage crops and vegetables. Legumes and cereals were the most common preceding crops, representing 50% and 25% respectively in durum wheat fields and 93% and 7% in soft wheat. The soil was ploughed (93% of fields), and plant residues were normally buried (≈85%). Soil fertilization was seldom practiced for organic durum (16%) and soft (30%) wheat.

In 2010, anthesis was from May 2nd to 5th for durum wheat and from April 25th to May 15th for soft wheat; harvesting date ranged from July 3rd to 27th and from June 24th to July 14th for durum and soft wheat, respectively. Wheat yield was quite variable: the average for durum and soft wheat was 2.4 and 4.5 t/ha, respectively. In 2011, anthesis was from April 27th to May 4th for durum wheat and for soft wheat from April 25th to May 15th; harvesting date ranged from June 28th to July 18th and from June 16th to July 28th

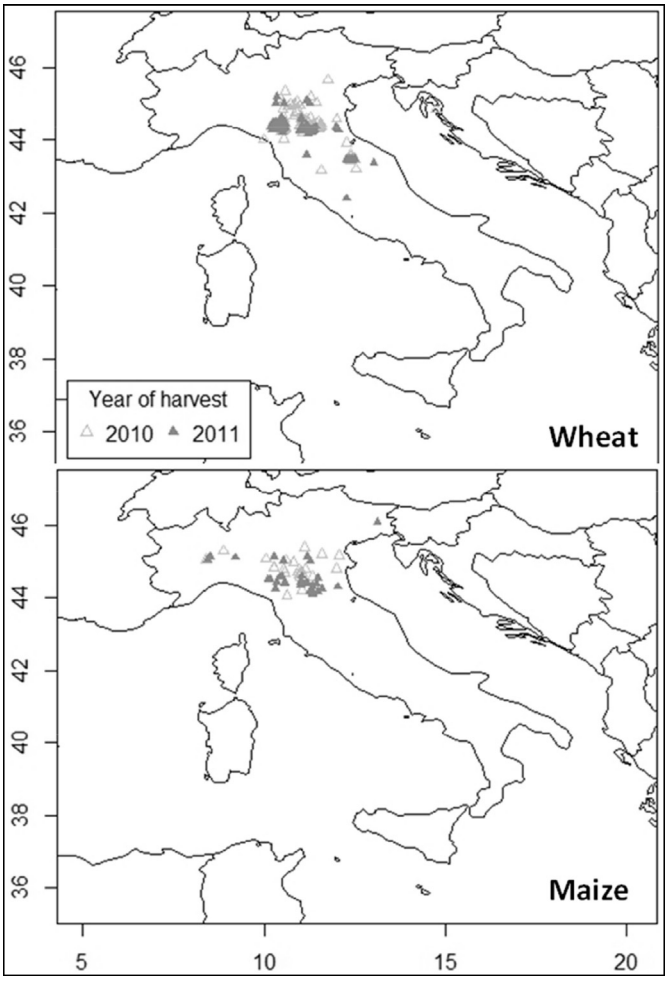

Fig. 1. Sampling locations of soft and durum wheat (a) and maize (b) from organic and conventional farming in 2010 and 2011.

for durum and soft wheat, respectively. Wheat yield was similar for durum wheat in both years, while it was higher for soft wheat in 2011 compared to 2010; the average yield was 2.6 and 6.0 t/ha, respectively.

3.1.2. Conventional wheat

Thirty-three samples of conventional durum (5) and soft wheat (28) were collected in the geographical area located between 44°22′27″–44°40′00″N, 11°17′15″–11°40′56″E and 44°20′50″–44°68′69″N, 10°53′00″–11°59′03″E, respectively (Fig. 1). Four varieties of durum wheat (sample number <5 each), and 9 varieties of soft wheat (Blasco the most used) were examined. Soft wheat varieties belonged to FP and FPS (≈76%) categories. Regarding crop rotation, industrial crops were the most used for durum wheat (40%), while cereals were the most utilized in soft wheat (48%). Soil fertilization was always practiced for both durum and soft wheat.

In 2010, anthesis was observed on May 2nd for durum wheat and occurred between April 26th and May 10th for soft wheat; harvesting period took place on June 29th for durum wheat and from June 26th to July 7th for soft wheat. The average wheat yield was 2.4 and 4.5 t/ha, for durum and soft wheat, respectively. In 2011, anthesis was observed from April 27th to May 3rd for durum and from April 25th to May 7th for soft wheat. The harvesting period was shorter than for organic wheat: from June 22nd to 25th for durum and from June 20th to 29th for soft wheat. Wheat yield was similar for durum and higher for soft wheat in 2011 compared to 2010; the average yield was 2.6 and 6.0 (t/ha) respectively.

3.1.3. Organic maize

Fifty-nine samples of organic maize were collected from a geographical area situated between 44°07′00″–46°08′33″N, 08°42′13″–13°11′05″E (Fig. 1). Thirty-eight hybrids were studied and only Agrister counted >5 samples. Preceding crops were mostly cereals (43%) and legumes (36%). The soil was ploughed (98.4% of fields), and plant residues were normally buried (≈85%).

In 2010, maize flowering occurred from June 25th to July 10th and harvesting lasted from September 10th to October 26th with an average relative humidity at harvest of 21.8%. In 2011 maize flowering occurred from June 15th to July 8th and harvesting lasted from August 7th to October11th, with relative humidity at harvest of 17.8% on average. ECB control was poorly carried out (3%).

3.1.4. Conventional maize

Ten samples of conventional maize were collected from a geographical area located between 44°32′17″–44°78′63″N, 11°32′04″–10°55′30″E (Fig. 1). Six hybrids were seeded. Crops preceding conventional maize were mostly cereals and maize (38% each).

In 2010, maize flowering occurred from June 26th to July 15th, harvesting lasted from September 18th to October 1st and the average relative humidity at harvest was 19.5%. In 2011, maize flowering occurred from June 10th to 18th; harvesting lasted from August 6th to September 2nd, with relative humidity at harvest of 17.1% on average. ECB control was practiced in less than 50% of the fields.

3.2. Meteorological data

3.2.1. Wheat

The year 2010 was characterized by variable weather conditions during wheat anthesis, in the two places considered as representative examples, Castelfranco Emilia and Medicina. Mean daily temperature varied between 13.5 °C and 19.5 °C during anthesis. Quite strong rainfalls were registered at the beginning of anthesis (from 8.5 to 12 mm per day) and rainy days were also registered at the end of anthesis (8 and 14.5 mm per day on average, respectively). Mean daily RH ranged between 56–90% and 48–90% in Castelfranco Emilia and Medicina, respectively (Fig. 2). Warm conditions were detected at harvest (Temperature Max = 29.5 °C; min = 19.6 °C, AVG = 25.3 °C), and dry days with light rainfalls at the end of the harvesting period, and RH ranging from 47.3% to 75.2%.

In 2011 mean daily temperature was 15.5 ± 2 °C during anthesis time and the weather was particularly dry with only two rainy days (5.2 and 14.5 mm per day in average) and RH around 70–80% (Fig. 2). The harvesting period was characterised by warm weather (Max = 28.6 °C; min = 20.0 °C, AVG = 24.5 °C), with very sporadic rainfalls and low average RH (41.5–77.1%).

3.2.2. Maize

Weather conditions in 2010 were comparable between the two places considered in the relevant period for maize. At early flowering temperature was on average 25 °C, with an average RH of 82%; then it decreased in correspondence with strong rainfalls,

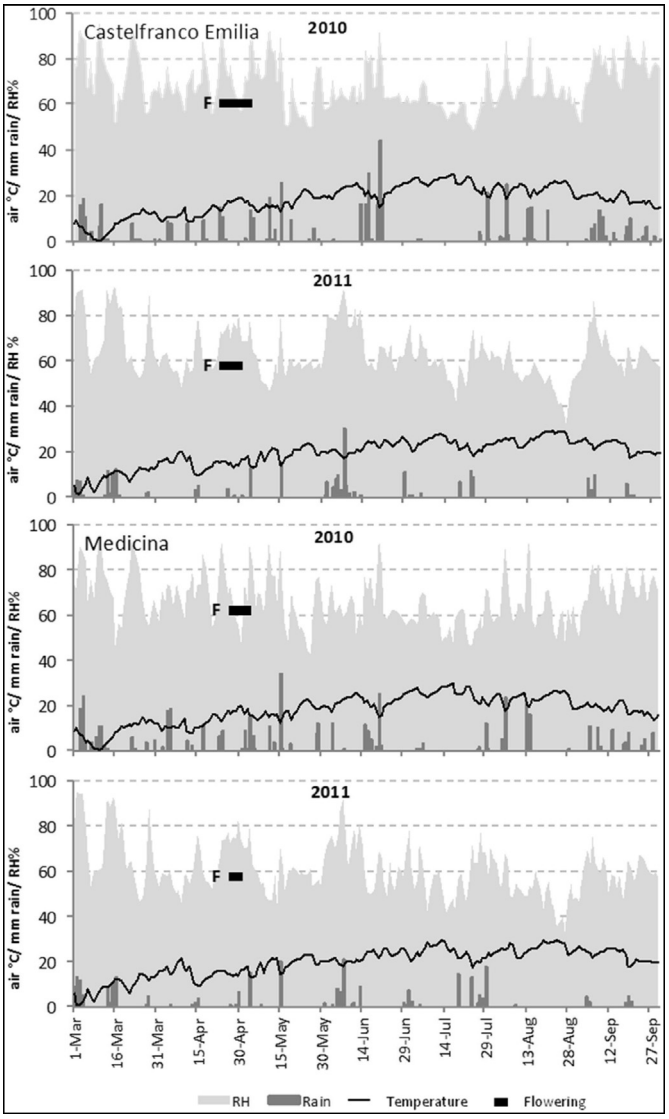

Fig. 2. Meteorological data [air temperature (°C), relative humidity (RH%) and rainfall (mm per day)] for 2010 and 2011 of two representative places for wheat cultivation: Castelfranco Emilia (MO) and Medicina (BO). F indicates the flowering growth stage and the black bar the interval of days when this growth stage was observed.

lasting a week in Mirandola (ranging from 17.1 to 43.9 mm), while it rained on only one day in Copparo (61.1 mm). The average RH reached 95.5%. During harvesting, it was not very warm (Max = 20.3 °C; min = 10.6 °C, AVG = 15.8 °C), particularly humid (RH = 59.9–90.6%) and rainy almost all the days, with peaks of 8 and 17 mm per day.

On the contrary, in 2011 the weather was very dry and characterised by warm temperatures (23.5 ± 2 and 21.5 ± 1.5, respectively in Mirandola and Copparo), and an initial RH = 76.5%, then decreasing to 60.5% on average for the rest of the growing period (Fig. 3). Also, during the harvesting period the weather conditions were very dry, with temperatures ranging from 18.0 (min) to 24.2 °C (Max) and RH = 49.2–70.8%.

3.3. Fungal isolation and morphological characterization

The fungal species isolated from wheat and maize and belonging to the mycotoxigenic genera *Aspergillus*, *Fusarium*, and *Penicillium* are summarized in Tables 1 and 2. However, kernels were mainly infected by other fungal species belonging to the genera *Alternaria*, *Aureobasidium*, *Cladosporium*, *Epicoccum*, *Mucor* and *Rhizopus*. The species of *Fusarium* isolated were: *F. graminearum*, *F. poae*, *F. proliferatum*, *F. subglutinans* and *F. verticillioides*.

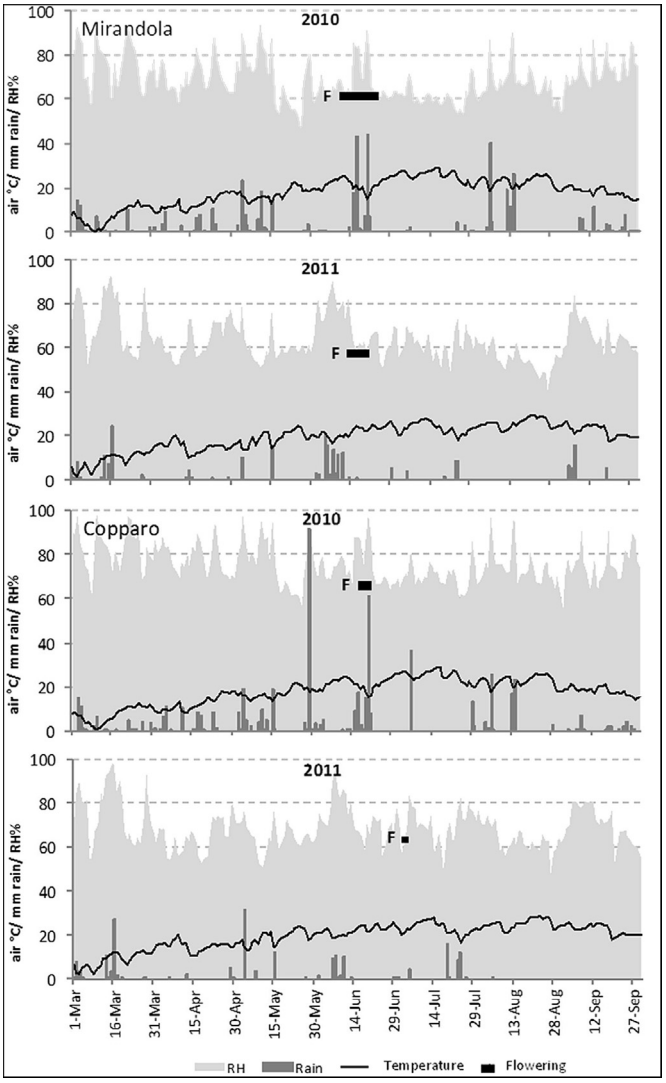

Fig. 3. Meteorological data [air temperature (°C), relative humidity (RH%) and rainfall (mm per day)] for 2010 and 2011 of two representative places for maize cultivation: Mirandola (MO) and Copparo (FE). F indicates the flowering growth stage and the black bar the interval of days when this growth stage was observed.

The ANOVA showed that the production system (organic *versus* conventional) and/or year of harvesting caused significant differences in both wheat and maize contamination by *Fusarium*, while no effect related to the wheat species grown (durum *versus* soft wheat) was recorded (Tables 3 and 4). In particular, the cropping system significantly influenced the incidence of *Fusarium* and *Aspergillus* in wheat: the conventional system was the most contaminated by *Fusaria* while the organic system had the highest contamination of *Aspergilli*. This significant effect was confirmed in maize only for *Fusaria*, with a higher incidence in organic crops. Regarding the growing year, a significant effect was noticed for fungal incidence ($P \leq 0.05$) in wheat, for *Fusarium* spp. and *Aspergillus* spp., and, in particular, at species level, for *F. graminearum* ($P \leq 0.01$). The scenario was similar in maize, where *Fusarium* spp., *Aspergillus* spp. and all the three *Fusarium* species found were significantly influenced. Evaluated as a whole, the considered factors were significant only for *Fusarium* and *Aspergillus* in wheat and for *F. verticillioides* in maize. Nevertheless, because of the relevant differences in the number of fields considered in organic and conventional farming, a detailed description of all the conditions studied was reported.

3.3.1. Organic soft wheat

The fungal incidence observed in organic soft wheat kernels ranged from 68% to 100%, in 2010, and 78%–100%, in 2011; the percentage of incidence ascribable to *Fusarium* spp. was 8.7% and 0.7%, respectively. On the other hand, neither *Aspergillus* spp. nor *Penicillium* spp. were isolated in 2010, while their incidence was 3% for both genera in 2011 (Table 1). With respect to the identification at species level of all *Fusarium* isolates, in 2010 *F. graminearum* was the most abundant (161 isolates, 70% of total *Fusarium* isolates), followed by *F. poae* (64 isolates, 28%, Fig. 4). The remaining 2% was represented by *F. proliferatum* with 4 isolates. In 2011, *F. poae* was the predominant species, accounting for 62% of *Fusaria* (21 isolates) *vs* 38% (13 isolates) of *F. graminearum* (Fig. 4).

3.3.2. Conventional soft wheat

Fungal incidence in conventional soft wheat ranged from 96% to 100% in 2010 and from 84% to 100% in 2011. *Aspergillus* and *Penicillium* isolates were not recovered in 2010, and only in low percentages in 2011 (1.4% and 0.9% of total fungal incidence, respectively, Table 1). *Fusarium* spp. occurrence was slightly higher than in organic soft wheat with 10.8% of total incidence in 2010, and 1.5% in 2011 (Table 1). Of the total *Fusarium* isolates, the species identified in 2010 were *F. poae* (52%) and *F. graminearum* (48%), while in 2011 *F. poae* (90%) and *F. subglutinans* (10%, Fig. 4) were found.

3.3.3. Organic durum wheat

Organic durum wheat showed a fungal incidence that ranged from 86% to 100% in both years (Table 1). However, species belonging to *Aspergillus*, *Fusarium*, and *Penicillium* were not isolated in 2010, while in 2011 *Fusarium* and *Penicillium* species were below 1% of isolation, and the fungal isolates identified as *Aspergillus* spp. were 25% of total fungal incidence (Table 1). The *Fusarium* species isolated in 2011 were only *F. poae* and *F. graminearum*; however, they occurred rarely (3 and 1 isolates, respectively) (Fig. 4).

3.3.4. Conventional durum wheat

Only one sample of conventional durum wheat was collected in 2010 and four in 2011. In the 2010 samples fungal incidence was 100%. No isolates belonging to *Aspergillus* spp. or *Penicillium* spp.

Table 1
Fungal incidence (*Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp.) in organic and conventional soft and durum wheat in 2010–2011.

Wheat type	Year	Samples (total)	Fungal incidence (%)			*Fusarium* spp. incidence (%)			*Aspergillus* (%)	*Penicillium* (%)	Other (%)
			Min	Max	Avg	Min	Max	Avg			
Organic soft	2010	72	68	100	*96.2*	2	48	*8.7*	0	0.3	87.2
Conventional soft		15	96	100	*99.7*	2	32	*10.8*	0	0	88.9
Organic durum		13	86	100	*96.3*	0	0	*0*	0	0.2	96.1
Conventional durum		1	–	100	*100*	–	14	*14.0*	0	0	86.0
Organic soft	2011	110	78	100	*95.8*	0	10	*0.7*	3.0	3.0	89.1
Conventional soft		13	84	100	*96.8*	0	6	*1.5*	1.4	0.9	93.0
Organic durum		11	86	100	*95.5*	0	4	*0.9*	25.4	0.2	69.0
Conventional durum		4	84	100	*93.0*	0	2	*0.5*	1.0	0	91.5

Table 2
Fungal incidence (*Fusarium* spp., *Aspergillus* spp. and *Penicillium* spp.) in organic and conventional maize in 2010–2011.

Maize type	Year	Samples (total)	Fungal incidence (%)			*Fusarium* spp. incidence (%)			*Aspergillus* (%)	*Penicillium* (%)	Other (%)
			Min	Max	Avg	Min	Max	Avg			
Organic	2010	24	16	100	*74.8*	8	96	*47.3*	3.6	2.4	24.1
Conventional		6	20	100	*60.7*	10	42	*29.0*	6.5	5.3	26.7
Organic	2011	35	22	100	*62.2*	2	82	*27.1*	1.3	0.9	32.9
Conventional		4	52	98	*72.0*	0	24	*7.5*	1	0	63.5

Table 3
ANOVA of the effects of production system and year of harvesting in fungal incidence on wheat.

Factors	Fungal incidence	*Fusarium* incidence	*Aspergillus* incidence	*Penicillium* incidence	*Fgram*[a]	*Fpoae*[a]
Production system	ns	**	**	ns	ns	ns
Organic	96.0	4.0	2.7	1.6	26.0	20.3
Conventional	97.7	6.0	0.5	0.4	15.1	29.4
Year	*	**	**	ns	**	ns
2010	96.7	8.0	0	0.3	23.9	14.6
2011	95.7	0.8	4.7	2.5	25.0	47.0

F. graminearum and *F. poae* were reported as a percentage of total *Fusarium* spp. isolated.
Regarding wheat type, "soft" and "durum" were not separated because they were not significantly different.
* = $P \leq 0.05$.
** = $P \leq 0.01$.
[a] *Fgram* = *F. graminearum*; *Fpoae* = *F. poae*.

Table 4
ANOVA of the effects of production system and year of harvesting on fungal incidence in maize.

Factors	Fungal incidence	*Fusarium* incidence	*Aspergillus* incidence	*Penicillium* incidence	*Fvert*[a]	*Fprol*[a]	*Fsubg*[a]
Production system	ns	**	ns	ns	*	ns	ns
Organic	67.3	35.3	2.2	1.5	55.2	29.9	9.5
Conventional	65.2	20.4	4.3	3.2	55.5	22.7	12.9
Year	ns	**	*	ns	**	**	*
2010	71.9	43.6	4.2	3.0	81.8	2.0	4.1
2011	63.2	25.1	1.3	0.8	34.2	50.2	14.5

F. verticillioides, *F. proliferatum* and *F. subglutinans* were reported as a percentage of total *Fusarium* spp. isolated.
* = $P \leq 0.05$.
** = $P \leq 0.01$.
[a] *Fvert* = *F. verticillioides*; *Fprol* = *F: proliferatum*; *Fsubg* = *F. subglutinans*.

were recovered (Table 1). *Fusarium* spp. were isolated in 14% of the kernels, with *F. graminearum* (3 isolates) and *F. poae* (2 isolates) identified (Fig. 4). In 2011, the fungal presence ranged from 84% to 100%, with a very low incidence of *Aspergillus* spp. (1%) and one *F. poae* (Fig. 4). No *Penicillium* spp. occurred in 2011 samples.

3.3.5. Organic maize

The incidence of infected kernels ranged from 16% to 100% in 2010 and from 22% to 100% in 2011 (Table 2). The recovery rate of *Aspergillus* and *Penicillium* species was lower than 2% of fungal incidence in both years, while *Fusarium* spp. incidence was higher in 2010 than 2011 with 47% and 27% of total fungal incidence, respectively (Table 2). Amongst all *Fusarium* species isolated, in the first year *F. verticillioides* was the most abundant (95%) and *F. subglutinans* and *F. proliferatum* were rarely recovered (Fig. 5). In the second year, *F. proliferatum* was the most abundant (52%), followed by *F. verticillioides* (37%) and *F. subglutinans* (10%) (Fig. 5).

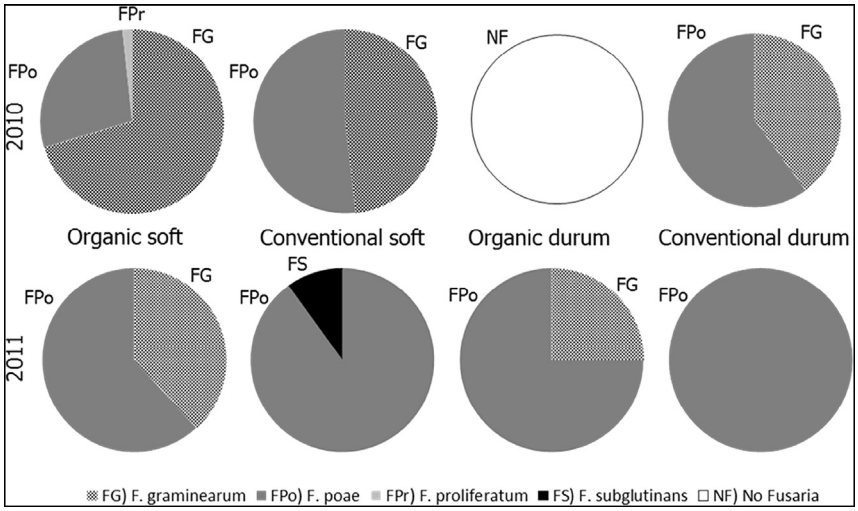

Fig. 4. Partitioning of *Fusarium* species isolated from organic and conventional soft and durum wheat sampled in the growing season 2009–2010 and 2010–2011 computed on the total.

3.3.6. Conventional maize

Fungal incidence varied between 20% and 100% in 2010 and 52% and 98% in 2011 (Table 2). *Aspergillus* spp. and *Penicillium* spp. were poorly recovered in 2010 and they were absent in 2011, while *Fusarium* spp. incidence was lower than in organic maize, being 29% as a mean of total fungal incidence in 2010 and 7.5% in 2011 (Table 2). *F. verticillioides* was the predominant species among the total *Fusarium* isolates in 2010 (89%), followed by *F. subglutinans* (6%) and *F. proliferatum* (5%), while in 2011 *F. proliferatum* was the most abundant (40%), with also high percentages of isolates identified as *F. verticillioides* (33%) and *F. subglutinans* (27%, Fig. 5).

3.4. Molecular identification of Fusarium species

The molecular analyses performed on the isolates randomly chosen, and representing all *Fusarium* species isolated in this study (*F. graminearum*, *F. poae*, *F. proliferatum*, *F. verticillioides* and *F. subglutinans*), confirmed the identification results obtained by using the morphological approach.

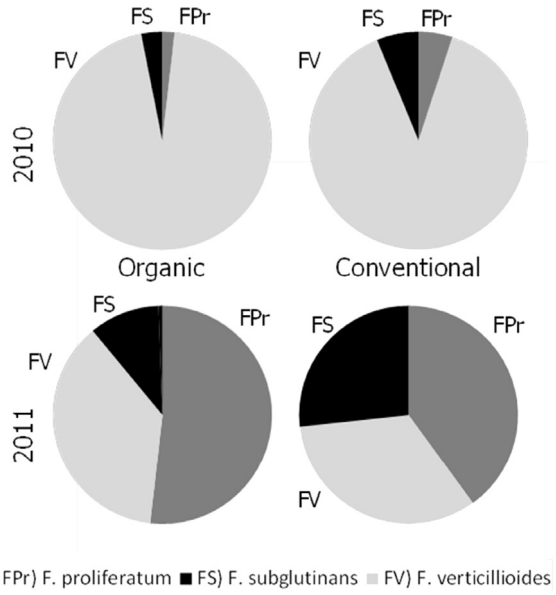

Fig. 5. Partitioning of *Fusarium* species isolated from organic and conventional maize sampled in the growing season 2010 and 2011.

4. Discussion

This study focused on the comparison between the incidence of mycotoxin-producing fungi in organic and conventional cropping systems applied in wheat and maize. Differences in fungal incidence were registered; interestingly, it was observed that contamination by *Fusarium* spp. was higher in conventional than organic wheat, independently of species, soft or durum (with the exception of durum wheat harvested in 2011). This finding agrees with Bernhoft et al. (2010), who observed a significantly higher incidence of *Fusarium* spp. occurring in conventional Norwegian cereals, including wheat, compared to organic crops. The higher *Fusarium* incidence in conventional wheat could be due to the use of pesticides not active against *Fusaria*, and mineral fertilizers (the main difference between the two farming systems) as previously demonstrated in other studies (Henriksen and Elen, 2005; Lemmens et al., 2004). The application of fungicides against leaf diseases on wheat, normally distributed before anthesis, could favour the spread of FHB, as the saphrofitic microflora on grains is suppressed and *Fusaria* find a competitive advantage (Lemmens et al., 2004). Furthermore, Henriksen and Elen (2005) proposed that fertilization with nitrogen in wheat resulted in an increase in crop density and an alteration of the canopy microclimate, resulting in higher humidity and therefore more favourable conditions for *Fusarium* infection.

On the other hand, we found that organic maize was more contaminated by *Fusarium* spp. than conventional maize in both years considered. This is partially in agreement with a Spanish study (Ariño et al., 2007) reporting that organic maize showed a higher total fungal incidence, but a lower *Fusarium* contamination of kernels compared to conventional maize. However, from the results of both studies we could perceive that, in general, organic maize is more prone to fungal attack, possibly due to a lack of chemical application. Insecticides demonstrated their efficacy because of the crucial role played by larvae in enhancing kernel infection (Mazzoni et al., 2011). The two other toxigenic genera, *Aspergillus* and *Penicillium*, occurred in maize at very low incidence in this study, and most probably those fungi did not compete with *Fusarium* in the environmental conditions observed during 2010

and 2011 in Italy.

In general, the total fungal population isolated from wheat and maize was high, although, considering the three main toxigenic genera, *Fusarium* represented a low percentage, especially in wheat, and the presence of *Aspergillus* and *Penicillium* was negligible.

Obvious influencing factors were the meteorological conditions of each year of crop growing, which were significant key points for fungal growth. Weather conditions were very important for the higher incidence of *Fusarium* species in 2010 *versus* 2011, for both conventional and organic farming, especially for soft wheat. The incidence of *F. graminearum* in conventional soft wheat was around 34% in 2010 while it was absent in 2011 on the same host. Differences in weather conditions were mainly observed during anthesis, while they were comparable at harvest time in 2010 and 2011. This confirms the relevance of conducive weather conditions in this plant stage to ensure infection efficacy (Parry et al., 1995). *F. poae* appeared the predominant *Fusarium* species in conventional wheat, according to some recent studies investigating the FHB species infecting kernels of organic (Infantino et al., 2012) and conventional soft (Pancaldi et al., 2010; Xu et al., 2008), and durum (Somma et al., 2010) wheat in Italy. Moreover, 2011 was a really favourable year for *F. poae* in all the types of wheat considered in this work. Previous studies showed that the presence of *F. poae* was associated with drier/warmer conditions, while *F. graminearum* was favoured by wetter/warmer conditions (Xu et al., 2008). Moreover, Lori et al. (2003) showed that a positive correlation existed between *F. graminearum* incidence and rainfall during the flowering period on durum wheat, in Argentina. Similar information was provided by Shah et al. (2005) for conventional wheat, in Italy. Regarding this study, the meteorological conditions in 2011 were drier than in 2010, with almost no rain, and RH ranging around 70–80% during flowering time confirming the key role played by meteorological conditions a for *Fusarium* species dominance, according to the above cited literature.

Maize was more infected by *Fusarium* species in 2010 than in 2011, in both organic and conventional crops. The influencing factor was the weather, which in the first year was colder and wetter and therefore more conducive for growth and development of *Fusarium* species. In particular, in 2010 the main fungal species recovered was *F. verticillioides*, for both farming systems considered, while in 2011 *F. proliferatum* predominated. *F. verticillioides* is considered to play a major role in maize pink ear rot and usually it is associated with *F. proliferatum* in a ratio of around 2:1 or higher (Fandohan et al., 2005; Logrieco et al., 1995). The two species have almost identical needs for growth (25 °C) and spore germination (30 °C), as optimum, with the only slight difference that the spores of *F. verticillioides* germinate with a wider range of temperature and water activity than *F. proliferatum* (Leslie and Summerell, 2006). *F. verticillioides* showed a significantly better growth *in vitro* at higher temperatures and water stress than *F. proliferatum* (Marín et al., 2010). This could explain the ability of *F. verticillioides* to colonize maize kernels at higher incidence in dry and hot regions (Logrieco and Moretti, 2008). However, in this study, the highest presence of *F. proliferatum* occurred in 2011, when environmental conditions were the driest, with mild and constant temperatures. Therefore, the reason for its predominance could be related to other factors than weather. A high predominance of *F. proliferatum* on maize kernels in the Northern-Central area of Italy was also reported by Logrieco et al. (1995), but no meteorological data were provided in that study and no comparison is therefore possible with this study.

Our data also showed important differences in the occurrence of *Aspergillus* and *Penicillium* spp. These fungi had a very low incidence in 2010, while a significant occurrence was found in 2011. These differences, especially those regarding *Aspergillus flavus*, could be attributed to the lack of rain during and after maize flowering in 2011. Indeed, *Aspergillus* spp., and in particular, *A. flavus*, have wind-borne spores not detected during rainy days (Battilani et al., 2013). Therefore, the dry conditions that occurred in 2011 could have improved *A. flavus* competitiveness against *Fusarium* species, increasing its occurrence in maize cultivated in both farming systems.

Conventional farming was significantly correlated with a higher and lower *Fusaria* incidence in wheat and in maize, respectively. Therefore, the use of pesticides and mineral fertilizers, associated with conventional farming, seems to be not as effective in the reduction of fungal incidence for wheat as it is for maize. The control of ECB, applied in many conventional fields, should be a key factor since the pest is known to ease *F. verticillioides* invasion in maize through the wounds made in the kernels (Munkvold et al., 1997). Several studies linked the control of ECB to the reduction of maize ear rot by decreasing fungal inoculum in maize kernels (Blandino et al., 2009; Mazzoni et al., 2011; Munkvold, 2003). On the other hand, nitrogen fertilisation is also a relevant factor for reducing fumonisin contamination in maize, since its poor availability causes high stress for many plants (Ariño et al., 2009; Marocco et al., 2008).

Crop debris, and specifically maize stalk residues, is known to be a source of inoculum for *Fusarium* species (Battilani et al., 2003; Cotten and Munkvold, 1998) and their burial and removal are good agricultural practices (Ariño et al., 2009). In this study, maize debris was commonly buried in organic fields, but is not in conventional farming. Therefore, the demonstrated higher concentration of Fusaria in organic maize might not be related to the difference in management of crop residues. On the other hand, this confirms that the limited control of insects in organic maize plays a key role, since they contribute by giving a crucial route of infection by injuries caused on the maize kernels, and also acting as vectors of fungal spores (Munkvold, 2003).

Other agricultural practices, such as crop rotation and soil tillage can help in reducing *Fusarium* incidence and the development of related diseases both in wheat (Dill-Macky and Jones, 2000) and maize (Lipps and Deep, 1991) fields. In this study, the type of crop rotation applied was comparable, for each crop, both in organic and in conventional fields, therefore not contributing to the differences observed. However it was demonstrated that these practices can act in a synergistic or additive way together with chemical fertilisation and pesticide usage (Edwards, 2004), thus further analysis would be needed to clarify a possible combined effect.

In conclusion, the comparison of conventional and organic farming showed few differences in the incidence of mycotoxigenic fungi infecting Italian wheat and maize in the two years considered. Conventional farming seemed to disadvantage wheat that was more prone to *Fusarium* infection, in particular to *F. poae*. On the other hand, conventional farming appeared to be safer for maize, which was less infected by *Fusarium* species, in particular *F. proliferatum*. The difference in the incidence of fungal species between the 2 years was much more relevant than between the two farming systems. This confirms that, for fungal development on cereals, a crucial role is played by meteorological conditions and this underlines the importance of weather monitoring as future climatic changes can dramatically change the distribution and the profile of toxigenic fungi on cereals worldwide.

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