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Integrated strategies for the control of Fusarium head blight and deoxynivalenol contamination in winter wheat.

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Abbreviations: FHB, Fusarium head blight; DON, deoxynivalenol; GDDs, growing degree days; GS, growth stage; MR, moderately resistant cultivar; S, susceptible cultivar.

Abstract

Fusarium head blight (FHB) disease and deoxynivalenol (DON) contamination of wheat grains depend on multiple factors, above all climatic conditions, but also agronomic factors such as crop rotation, debris management, variety susceptibility and fungicide applications. Although it is generally believed that multiple strategies are more successful than a single strategy, only a few studies have shown the quantitative effect of combining multiple strategies.

Field experiments have been conducted over three growing seasons in three sites in Northern Italy to evaluate the effect of previous crop residue management through tillage, variety susceptibility and triazole fungicide application on common wheat, according to a full factorial scheme. The following parameters were analyzed: FHB severity, grain yield and DON contamination.

The collected data have clearly shown a close interaction between the factors involved in FHB severity and DON content, while the interactions were less significant for grain yield. In all nine trials, the DON contamination was significantly affected by the interaction of at least two of the compared factors, while the interaction between all three factors involved was significant in four trials. The most favourable scenario to avoid DON contamination (ploughing, moderately resistant variety, triazole application at heading) reduced the DON content by 97% compared to the worst one (direct sowing, susceptible variety, no fungicide application).

Since the interaction between the agricultural practices have shown a synergistic effect, integrated multiple strategies, in areas characterized by a high risk of FHB, can be considered the very effective management means of reducing FHB and DON contamination in wheat.

Keywords: winter wheat, residue management, variety susceptibility, fungicide application, Fusarium head blight, deoxynivalenol.

1. Introduction

Fusarium Head Blight (FHB) is the most diffuse wheat ear disease throughout the world and it is caused by *Microdochium nivale* and different *Fusarium* species (Champeil et al., 2004a). This disease causes total or partial ear premature senescence with a consequent reduction in both crop yields and grain quality (Pirgozliev et al., 2003). *F. graminearum* and *F. culmorum*, the most important species responsible for FHB, are also the main causes of the accumulation of deoxynivalenol (DON) in wheat kernels, a mycotoxin of the trichotecenes group, inhibits protein biosynthesis in eukaryotes (Bottalico and Perrone, 2002).

FHB infection and DON contamination of wheat grains depend on multiple factors, above all climatic conditions, particularly at flowering (Xu, 2003), but also agronomic factors such as crop rotation, debris management, variety susceptibility and fungicide applications (Pirgozliev et al., 2003; Koch et al., 2006), which aim at reducing infection or growth of toxigenic fungi (Aldred and Magan, 2004).

The primary reservoir of inoculum is debris from the previous crop (Xu, 2003). FHB epidemics are supported by cropping systems that leave high amount of crop debris on the soil surface (Pereyra and Dill-Macky, 2008; Blandino et al., 2010) and pathogens survive longer on residues that do not degrade easily, such as stem nodes or stalks (Sutton, 1982). Thus, FHB disease and DON contamination are more severe if the preceding crops are maize or sorghum, rather than wheat or barley and even less contamination is observed following other crops (Champeil et al., 2004b; Smith-White et al., 2004).

Limited soil tillage or no-tillage increase the frequency of FHB, whereas deep tillage, such us ploughing, decreases it (Miller et al., 1998). Maiorano et al. (2008) reported a close relationship between DON contamination in wheat grains and the quantity of maize crop residues on the soil surface at anthesis. Moreover, FHB severity and DON content are clearly affected by the interaction of previous crop residues and tillage practice applied (Dill-Macky and Jones, 2000).

As far as variety susceptibility to FHB and DON is concerned, breeding progress in cereals, using conventional methods, molecular markers or through transgenic approaches, have been discussed in great detail in several reviews (Hollins et al., 2003; Snijders, 2004). At present, no durable, fully FHB-resistant wheat cultivars exist, therefore their control relies on the use of commercial cultivars with partial resistance (Mesterhàzy et al., 2005), although wheat varieties more resistant to FHB have been shown to reduce DON production to almost nil in recent studies (Tóth et al., 2008).

The effect of fungicide application on FHB and DON contamination control has been well documented. Several studies conducted on *in vitro* experiments (Ramirez et al., 2004), on field trials in which wheat was artificially inoculated (Mesterhazy et al., 2003; Chala et al., 2003) or under natural infection conditions (Blandino et al., 2006) have demonstrated that good levels of control can be achieved with fungicides. The outcome of the use of fungicides seems to depend on the fungal species that are present and the effect that the particular fungicide has on these species. Fungicides containing triazole, imidazole or triazolinthione active ingredients, which inhibit the biosynthesis of ergosterol, were the most active against FHB infection and DON contamination (Haidukowski et al., 2005; Ioos et al., 2005). Of the azole group, metconazole and prothioconazole, which have been developed more recently, have been reported to be the most effective fungicides in controlling *Fusarium* spp. and reducing the DON level in wheat grain (Paul et al., 2008).

Previous studies show that individual control methods can decrease the impact of the disease significantly, but combining control methods can be expected to be more efficient, especially if the climatic conditions are favourable for FHB infection (Edwards, 2004). Therefore good agricultural practice (GAP) requires an integrated approach that addresses all the possible risk factors in order to prevent DON contamination (Pirgozliev et al., 2003). Moreover, although information is available on the basic effect of individual agricultural practices on *Fusarium* infection and DON contamination in wheat, only a few studies have been conducted to quantify the relative importance of each of these factors compared to the others or to verify their interactions and combined effects.

The aim of this study was to determine the effect of residue management, variety susceptibility and fungicide application on FHB infection and DON contamination in wheat kernels, with a particular attention to their interactions under natural infection conditions.

2. Materials and Methods

2.1. Experimental site and treatments

The experiments were carried out between 2005 and 2008 at 3 sites in North Italy: Imola (IM), Riva presso Chieri (RC) and Sant'Angelo Lodigiano (SL). The geographic and the main agronomic information about the experimental fields is reported in Table 1.

The compared treatments were factorial combinations, in natural conditions, of:

- The previous crop residue management through tillage: ploughing to a 30 cm depth, thus incorporating the debris in the soil, followed by disk harrowing to prepare a proper seedbed vs. direct sowing;
- variety susceptibility: a variety classified as moderately resistant (MR) to FHB infection and DON contamination vs. a susceptible (S) one;
- fungicide application: a triazole fungicide application at heading [growth stage (GS) 59] (Zadoks et al., 1974) vs. an untreated control.

The treatments were assigned to experimental units using a split-plot design, with the previous crop residue management as the main-plot treatment and the variety susceptibility and fungicide application as the sub-plot treatments. Each trial was replicated three times in the IM and SL sites and four times in the RC site. The sub-plot was 7 x 2 m.

The previous crop was grain sorghum at site A (growing seasons 2005-06 and 2006-07) and grain maize at site A (2007-08), B and C. Since maize and sorghum are the most dangerous previous crops in the context of FHB epidemics and DON contamination (Gourdain, 2008), they have been selected to verify the effect of residue management through tillage in the more risky crop rotation conditions.

The MR variety that was used in each year and site was cv. Bologna , while cv. Serio was the S one (Mayerle et al. 2007).

In each trial, the triazole fungicide was metconazole (Caramba®, Basf, Italy, formulation: suspension concentrate) and it was applied at 0.06 kg active ingredient (AI) ha^{-1}. The fungicide was applied with a 4 nozzle precision sprayer (T-Jeet 110/04) using a fine mist at a slow walk to ensure an effective coverage. The delivery pressure at the nozzle was 324 KPa.

Planting was conducted in 12 cm wide rows at a seeding rate of 450 seeds m^{-2}. The weed control was conducted with isoproturon and diflufenican at wheat tillering (GS 31). Glyphosate was applied to the non tilled field before direct sowing. A total of 140 kg N ha^{-1} was applied to plots as a granular ammonium nitrate fertilizer, and was split equally between GS 31 and 39. The sowing, fungicide application and harvesting date for each year and each site are reported in Table 1.

Grain yields were obtained by harvesting with a Walter Wintersteiger cereal plot combine-harvester. The grain yield results were adjusted to a 120 g kg^{-1} moisture content. The harvested grains were accurately mixed, and 2 kg grain samples were taken from each plot to analyse the DON content.

2.2. FHB symptom evaluation

FHB severity was recorded for each plot at the soft dough stage (GS 85) by carrying out visual evaluations of the disease. FHB severity was computed as the percentage of spikelets per ear with symptoms. A scale of 1 to 7 was used in which each numerical value corresponds to a percentage interval of surfaces exhibiting visible symptoms of the disease according to the following schedule: 1 = 0-5%, 2 = 5-15 %, 3 = 15-30%; 4 = 30-50 %, 5 = 50-75%, 6 = 75-90%, 7 = 90-100% (Parry et al., 1995). The scores were converted to percentages of the ear exhibiting symptoms and each score was replaced with the mid-point of the interval.

2.3. DON analyses

A 2 kg representative sample of wheat kernels from each plot was finely ground using a Model MLI 204 Bühler laboratory mill (Bühler S.p.A, Milan, Italy) to pass a 1 mm sieve. The DON concentrations were determined according to the method reported by Neumann et al. (2009) on the basis of an immunoaffinity column clean-up of the extracts, and mycotoxin was determined by means of HPLC/UV.

Briefly, 25 g of ground samples were added to polyethylene glycol (PEG-8000) and extracted with water by blending. Extracts were filtered through filter paper (Whatman no. 4) and glass microfibre filter (Whatman GF/A) and cleaned up by DONTest immunoaffinity column (VICAM, Milford, MA, USA). The toxin was determined by reversed-phase HPLC apparatus with a diode-array UV detector set at 220 nm (1100 Series HPLC Value System, Agilent Technologies Inc., Santa Clara, CA, USA) . The column was a Synergi 4 µm Hydro RP 80A, 150 × 3 mm (Phenomenex, Torrance, CA, USA). The mobile phase consisted of a mixture of acetonitrile:water (10:90) eluted at a flow rate of 0.5 mL min^{-1}. Recovery experiments were performed in triplicate using DON free wheat samples spiked at levels of 100, 500, 1000 and 2000 µg kg^{-1}. Recoveries were higher than 80% with relative standard deviations less than 10%. DON standard used for recovery experiments and HPLC calibration curves was purchased from Sigma-Aldrich s.r.l. (Milan, Italy).

Appropriate dilutions of the sample extracts contaminated with higher DON levels than 2000 μg kg^{-1} were necessary before loading them into the immunoaffinity columns in order to avoid saturation of the DON-antibody binding sites. The detection limit of the method was 20 μg kg^{-1} (signal-to-noise ratio of 3:1).

2.4. Statistical analysis

The effect of agronomic factors on FHB severity, grain yield and DON content was tested by means of a repeated measure analysis of variance (RM-ANOVA) in which the management of the previous crop residue was the between-subject factor, while the variety susceptibility and fungicide application were the within-subject factors. The residual normal distribution was verified using the Kolmogorov-Smirnov test, while variance homogeneity was verified using the Levene test. When the interactions between the factors were significant, the resultant means were compared using the protected Fisher Least Significant Difference (LSD) adjusted for multiple comparison using the Bonferroni procedure. The RM-ANOVA was conducted separately for all the year and site combinations, in order to verify clearly in each experiment the interactions between the involved agronomic factors. The statistical package SPSS for Windows, Version 17.0 (SPSS Inc., Chicago) was used for the statistical analysis.

3. Results

3.1. Weather conditions

The three growing seasons showed different meteorological trends from the beginning of the stem elongation stage to the harvest (Table 2). In 2006, the precipitations were not particularly elevated, but they were concentrated close to anthesis (GS 65), particularly at the IM and RC sites. In 2007, frequent rainfall occurred at the IM and RC sites, but only at the end of ripening (June), while the rainfall was higher from anthesis to the milk stage (GS 75) at the SL site. In 2008, instead, the precipitations were frequent and regular from April to June, above all from the beginning of flowering to the soft dough stage at the RC and SL sites, thus prolonging the harvest till the middle of July. In 2006 and 2007, the growing degree days (GDDs) were particularly high in June, thus quickening the canopy senescing process and leading to a reduction in the grain filling period and to an early maturity of the crop.

3.2. FHB severity

In eight of the nine trials, the FHB severity was significantly affected by the interaction of at least two of the compared factors, while the interaction between all three factors involved was significant in four trials: in 2006 at site IM, in 2007 at site SL and in 2008 at site RC and SL (Table 3).

In 2006, at site IM, when applied on their own in order to control FHB, none of the factors was able to reduce FHB severity compared to the worst scenario (direct sowing, S variety, no fungicide application), while a significant reduction in disease symptoms was always observed with the preventive combination of all the factors (ploughing, MR variety, fungicide application) (Table 4). In 2007, at site SL, the ploughing or the use of an MR variety, but not the use of a fungicide, significantly reduced FHB severity compared to the combination of direct sowing, S variety and no fungicide application; on the other hand, all the combinations of two factors in order to prevent FHB symptoms, reduced disease severity significantly more than the worst scenario. In 2008, at site RC, the application of one of the considered factors on its own to prevent FHB significantly reduced the disease severity compared to the worst scenario (direct sowing, S variety, no fungicide application). Moreover, a significant further reduction in FHB severity was obtained with a fungicide application to the MR variety in direct sowing conditions and to the S variety after ploughing, compared to the untreated control, or with an MR variety instead of the S one in the ploughed and untreated plot. In 2008, at site SL, ploughing and fungicide application, but not the adoption of the MR variety, were able to significantly reduce FHB severity compared to the worst scenario (direct sowing, S variety, no fungicide application). In both sites, no significant further reduction was observed, even for the best combination of the three factors.

3.3. Grain yield

Grain yield was affected significantly by the interaction of at least two of the factors compared in the trial conducted in 2006 at the SL site, in 2007 at the RC site and SL site (Table 5). In the other trials, the main effect of at least one of the factors resulted to be significant. Ploughing significantly increased grain yield compared to direct sowing in the trial conducted at the RC site by 17% in 2006 ($P<0.01$), by 26% in 2007

($P<0.01$) and by 62% in 2008 ($P<0.001$), and by 66% in 2008 at the SL site ($P<0.001$) (Table 6).

The MR variety was significantly more productive than the S one at the IM site in 2006 (7% more, $P<0.05$) and in 2007 (9% more, $P<0.01$) and at the RC site in 2006 (11% more, $P<0.01$).

The fungicide application at heading significantly increased the yield at the IM site ($P<0.01$) and the RC site ($P<0.01$), by 13% and 4%, respectively in 2006, at the IM site ($P<0.01$) and the SL site ($P<0.01$), by 19% and 14%, respectively in 2007, and at the IM site ($P<0.01$), the RC site ($P<0.001$) and the SL site ($P<0.01$), by 14%, 55% and 10%, respectively in 2008.

In 2006, at the SL site, the interaction between the three factors was significant ($P<0.01$): when applied on their own to control FHB, none of the factors was able to increase grain yield compared to the worst situation (direct sowing, S variety, no fungicide application), while a significant increase in yield of 27% was observed for the preventive combination of all the factors (ploughing, MR variety, fungicide application). The interaction between variety and fungicide was significant in 2007 at the RC site ($P<0.05$): when the fungicide was applied at heading, the S variety showed a significantly higher grain yield than the MR one. On the other hand, no differences were observed in the untreated conditions and the fungicide did not significantly increase the yield in either variety (Table 6). In 2007, at the SL site, the interaction between the tillage and variety was significant ($P<0.01$): both the ploughing and the use of an MR variety significantly increased grain yield compared to the combination of direct sowing and the S variety, while no significant further increase was observed for the combination of ploughing and the MR variety (Table 6).

3.4. DON contamination

The average DON content was clearly related to the meteorological conditions, particularly close to anthesis, in each year and site. The mean DON contamination was low (< 100 µg kg^{-1}) at the SL site in 2006 and at the IM site and the RC site in 2007. However, mean DON content was extremely high in 2008 at the RC (12995 µg kg^{-1}) and SL (9310 µg kg^{-1}) sites. In the other trials, the mean DON contamination was between 262 and 710 µg kg^{-1} (Table 8).

In all the trials, the DON contamination was significantly affected by the interaction of at least two of the compared factors, while the interaction between all the three factors involved was significant in four trials: in 2006 at SL site, in 2007 at RC site and in 2008 at IM and SL sites (Table 7). In 2006 at the SL site and in 2008 at IM site, when applied on their own to control FHB, all the factors were able to reduce DON contamination compared to the worst scenario (direct sowing, S variety, no fungicide application). Furthermore, no significant further reductions were observed, even for the best combination of the three factors (Table 8). In 2007, at the RC site, a significant difference was only observed between the best (ploughing, MR variety and fungicide application) and the worst scenario (direct sowing, S variety, no fungicide application). In 2008, at the SL site, the ploughing and the fungicide application, but not the MR variety, significantly reduced the DON content compared to the combination of direct sowing, S variety and no fungicide application. On the other hand, all the two-factor combinations to prevent FHB significantly led to a further reduction in DON occurrence. Compared to the best scenario (ploughing, MR variety, fungicide application), direct sowing or the adoption of the S variety significantly increased the DON contamination.

The reduction in DON level by means of a factor application (variety, tillage, fungicide) can be expressed by a parameter, efficacy (E), which is defined by the following ratio (Blandino et al., 2011):

$$E(\%) = \left[\frac{\text{(control DON level – treatment DON level)}}{\text{control DON level}} \right] \times 100$$

On average, the three investigated factors showed a different efficacy in reducing the DON content, when assessed separately in the trials with low (DON < 100 μg kg^{-1}), medium (100 < DON < 1250 μg kg^{-1}) or high (DON > 1250 μg kg^{-1}) FHB pressure (Table 8). The efficacy of the variety for medium and high disease pressure, were higher (77%) than in the trials in which the *Fusarium* infection was low (29%). The efficacy of the fungicides in reducing DON on average decreased moving from low (69%) to high (46%) FHB severity. The efficacy was higher for tillage (75%) for high and medium FHB pressure than trials with a low DON content (63%). As far as previous crop is concerned, the efficacy for tillage after sorghum was 83% (2006 and 2007, at site IM) and it was 68% after maize, in the other experiments.

The average data of the relative DON content for the different agronomic situations extrapolated from the experiments are reported in Figure 1. The data are expressed in relation to the worst possible case scenario (direct sowing, S variety, no fungicide) in each trial. When applied on its own, the management of the previous crop residue through tillage resulted to be the best agronomic practices to minimize the field contamination of this mycotoxin (-68%) compared to the worst scenario, and this was followed by the use of an MR variety (-61%) and the triazole application at heading (-41%). The effect of the combination of the compared factors to control DON was simulated: the individual efficacies (E) observed for each factor compared to the worst scenario were combined in an additive way. In the new scenarios, obtained by the introduction of a control factor, the DON content was calculated using the following equation:

$$S_{1,2} = S_1 – (S_1 * E_2 / 100)$$

where $S_{1,2}$ = DON content in the scenario which applies factors 1 and 2; S_1 = DON content in the scenario which applies factors 1, with the highest efficacy; E_2 = efficacy observed for factor 2 compared to the worst scenario.

The simulated results of the combination of each factor were compared with the average effective data observed in field trials. The combinations of avoided risk factors decreased the DON content in a synergistic manner, since the observed data always showed a greater reduction in DON than the simulated ones. The most favourable scenario for DON contamination (ploughing, MR variety, triazole application at heading) reduced the DON content by 97%.

4. Discussion

The results of these experiments, conducted over three years characterized by extremely different meteorological trends, confirm the significant link between agronomic practices and FHB infection and DON contamination.

The collected data clearly underline that previous crop residue management through tillage, wheat variety susceptibility and triazole application are important tools that can help growers minimize DON contents in wheat grain.

As proposed by Koch et al. (2006), information on the relative effect of management options on DON contamination can be obtained through a simplified approach that calculates the severity of the relative effect of individual factors. In this study, the severity of the effect of the individual agricultural practices was calculated as follows: the mean DON value of the treatment with the highest DON concentration divided by the mean value of the lowest treatment. The data obtained from our experiments are reported in Table 9 and compared with other data available from literature. In all these studies, the effect of at least two agricultural practices on DON contamination were compared, in naturally-infected field conditions. Based on these data, the main factors that influence DON formation in wheat grain can be put in a ranking order as follows: susceptibility of wheat variety (3.8) ≥ the preceding crop (3.1) > soil tillage (2.4) ≥ fungicide application at anthesis of wheat (2.3). Thus, DON control in wheat should start in the field, and should first focus on the agronomic factors that influence FHB infection. Above all, conditions such as preceding host crops, especially maize and sorghum, which leave high amount of infected residues in the field, and the cultivation of a susceptible variety contribute to heavy *Fusarium* infections of wheat crops. The raking order summarized from these first data obtained in non-inoculated trials, need to be confirmed for different environmental and management conditions from those here reported.

Our data clearly underline how the efficacy of agronomic practices on controlling DON is affected to a greater extent by the climatic conditions and different meteorological trends could therefore change the order of importance of the involved factors. Variety susceptibility plays a more important role for low or medium disease pressure, while, when high inoculums are present, as observed in our experiment in 2008 at the RC and SL sites, the difference between susceptible or moderately resistant varieties may not be significant. A clear interaction between climatic conditions and cultivar was observed concerning the composition of the FHB species on wheat heads in Germany (Klix et al., 2008). Schaafsma et al. (2001) reported a significant interaction between the effect of variety on DON levels and year: no difference was observed among varieties in the years when the meteorological trend was unfavourable for *Fusarium* infection.

On the other hand, the no tillage practice, which leaves crop residues unburied, clearly increased the DON contamination in all the trials. The effect of the presence of residues on the soil surface, increased the DON content much more when the climatic conditions were favourable, but not excessive, for inoculum production and spore dispersal. In a previous work (Blandino et al., 2010), it was shown that the effect of maize residue density on DON content is less evident in dry conditions during the susceptible stages of wheat development or with very frequent rainfall, which probably greatly disadvantages or advantages inoculum production, respectively. Lori et al. (2009) reported a significant difference between conventional and no tillage practices, but only when the weather conditions were moderate for FHB.

The fungicide treatment was the agricultural practice which showed the greatest grain yield advantage. Since the application of a fungicide from heading to anthesis is associated with yield increases, due to the maintenance of the photosynthetic life of the canopy during grain filling (Ruske et al., 2003), the effect of this agricultural practice on yield was also observed in the trials with low FHB pressure. Moreover, the effect of triazole fungicide application at heading on FHB and DON control has been pointed out in all the trials. The DON contamination was reduced by the fungicide to a greater extent in the trials with low FHB pressure than in those with high *Fusarium* infection. Mesterhazy et al. (2003) achieved a higher efficacy when the fungicides were applied to a moderately resistant cultivar rather than to a susceptible one, while McMullen et al. (2008) reported that the effect of a triazole application in reducing DON doubled when the previous crop was canola, which determines a clearly lower FHB infection, rather than wheat.

Overall, when the meteorological trend, particularly around wheat anthesis, does not lead to a high *Fusarium* infection, the DON content in the grains at harvesting is only significantly higher for a combination of several risk factors, while, in these conditions, the presence of an individual risk factor does not increase the contamination to any great extent. On the other hand, for climatic conditions that promote a high production of *Fusarium* inoculum and the consequent fungal infection and development on the wheat ears, the effect of different agronomic scenarios shows a greater impact on the final DON content.

Therefore, although the knowledge of the relative importance of the individual factors that influence DON formation is crucial for the development of decision support systems that aim at minimizing DON concentrations in wheat grain, our data have clearly shown a close interaction between the agronomic factors involved in FHB severity and DON content.

The collected data clearly underline that a combination of two or more agricultural practices in a integrated multiple management strategy can result in a better control of DON contamination.

As far as the maize residue level, fungicide application and cultivar resistance on DON concentrations in spring wheat is concerned, Nita et al. (2006) reported significant positive interactions between the compared agronomic factors in several cases. The highest grain yield and the lowest DON content in spring and winter wheat were also achieved with multiple, rather than single, management strategies by McMullen at al. (2008). Obst et al. (2000), in a 4-year study in Germany, determined four risk factors: (i) maize as the previous crop, (ii) minimum tillage after maize, (iii) use of a moderately or highly susceptible wheat variety and (iv) application of a strobilurin product. In this experiment, an individual risk factor increased the relative risk of DON contamination three-fold, while the combination of four risk factors increased the relative DON risk 56-fold. In France, ARVALIS, use a grid, derived from a 7-year study, to manage wheat lots during harvesting. This grid has 7 DON contamination risk levels, based on 3 combined risk factors: previous crop, tillage and varietal susceptibility (Gourdain, 2008). Comparing the most favourable and the worst DON control scenarios, Koch et al., (2006), reported, in a 2-year trial, which involved the same factors as our experiment, the same reduction in DON content (97%) observed in our work.

As far as our data are concerned, only in one of the nine field trials, was the application of the best integrated strategies not able to reduce DON contamination under the present EU admissible maximum levels for common wheat (i.e. 1250 μg kg^{-1}; EC 2006), but, it is important to underline that the experiment was conducted in climatic conditions that were extremely favourable for FHB infection and DON content.

Moreover, the data collected on the integrated multiple agronomic strategies clearly confirm the hypothesis advanced by Edward (2004) and Beyer et al. (2006), concerning the fact that the impact of combined risk factors on DON contamination is synergistic rather than additive.

In the future, other factors which have proven to have a possible, although often conflicting, effect on DON control in wheat, such as N fertilization (Lemmens et al., 2004), planting date and canopy density (Champeil et al., 2004a), weed control (Jenkinson and Parry, 1994), seed dressing with fungicide (Poels et al., 2006; Campagna and Fusarini, 2010) and biological control (Palazzini, 2007), need to be introduced into this integrated approach and tested to establish their impact on DON content.

In short, our results, which were obtained under naturally-infected field conditions, provide useful information to help measure the impact of previous crop residue management practices, variety susceptibility and triazole fungicide application, some of the most important practices adopted to control FHB in wheat, on grain yield and on DON contamination. Since the interaction between the agricultural practices have shown a synergistic effect, integrated multiple strategies, in the areas characterized by a probable FHB infection, would seem to be the best management way of reducing FHB and DON contamination in wheat.

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Table 1

Main trial information for the field experiments conducted in the 2005-2008 period in 3 sites in North Italy

Growing season	Site	IM	RC	
	Location	Imola (IM)	Riva presso Chieri (TO)	S. Ang
	Geographic coordinates	44° 21' N, 11° 42' E	44° 54' N, 7° 24' E;	45°
	Altitude (m)	21	262	
	Soil [(a)]	Silty-sandy-loamy, Vertic Haplustepts	Loamy, Aquic Frugiudalf	Sandy
2005-2006	Previous crop	Sorghum	Maize	
	Sowing date	22/11/05	28/10/05	
	Date of fungicide application	18/05/06	18/05/06	
	Harvest date	12/07/06	10/07/06	
2006-2007	Previous crop	Sorghum	Maize	
	Sowing date	18/10/06	27/10/06	
	Date of fungicide application	26/04/07	06/05/07	
	Harvest date	21/06/07	28/06/07	
2007-2008	Previous crop	Maize	Maize	
	Sowing date	22/10/07	02/11/07	
	Date of fungicide application	07/05/08	16/05/08	
	Harvest date	07/07/08	15/07/08	

(a) USDA classification

Table. 2

Monthly rainfall and growing degree days (GDD 0s) from March to July 2006-2008 in the research sites.

Year		2006			2007			
		Rainfall	Rainy days	GDD 0s[a]	Rainfall	Rainy days	GDD 0s[a]	Rainfall
Site	Month	(mm)	(d)	(°C d^{-1})	(mm)	(d)	(°C d^{-1})	(mm)
IM	March	49	18	256	70	16	317	54
	April	58	18	396	12	5	463	62
	May	54	15	532	63	12	579	70
	June	27	13	641	53	11	650	62
	April - June	188	64	1825	198	44	2008	248
RC	March	17	3	277	29	8	365	20
	April	21	8	430	13	3	509	105
	May	60	9	558	76	14	606	137
	June	19	6	679	107	12	667	97
	April - June	116	26	1944	225	37	2147	359
SL	March	23	5	275	42	8	335	31
	April	65	14	418	13	4	517	77
	May	49	12	565	101	11	603	96
	June	18	5	683	79	12	673	80
	April - June	156	36	1942	235	35	2127	283

(a) Accumulated growing degree days for each decade using a 0°C base.

Table. 3

Analysis of variance for FHB severity, field experiments conducted at three sites in North Italy over a three years period.

Site	Year / Source of variation	2006 P	2006 sem	2006 protected LSD	2007 P	2007 sem	2007 protected LSD	2008 P	2008 sem	2008 protected LSD
IM	Tillage (A)	0.020	0.16		0.012	0.03		0.014	0.18	
	Variety (B)	0.002	0.18		0.003	0.05		0.013	0.28	
	Fungicide (C)	0.031	0.37		0.029	0.07		0.001	0.40	
	A X B	0.003	0.26		0.004	0.07	0.47	0.243	0.40	
	A X C	0.042	0.52		0.065	0.10		0.021	0.56	3.86
	B X C	0.013	0.23		0.064	0.10		0.284	0.34	
	A X B X C	0.014	0.32	3.35	0.572	0.13		0.937	0.48	
RC	Tillage (A)	0.251	0.82		0.367	1.86		0.002	0.99	
	Variety (B)	< 0.001	0.93		0.208	2.31		< 0.001	1.66	
	Fungicide (C)	< 0.001	0.79		< 0.001	1.79		< 0.001	1.12	
	A X B	0.629	1.32		0.611	3.27		0.038	2.34	
	A X C	0.938	1.12		0.230	2.53		< 0.001	1.59	
	B X C	0.001	1.17	6.37	0.793	2.10		< 0.001	1.40	
	A X B X C	0.923	1.65		0.653	2.96		0.008	1.98	14.89
SL	Tillage (A)	0.004	0.13		0.018	0.87		0.012	1.72	
	Variety (B)	< 0.001	0.12		0.001	1.55		0.751	2.27	
	Fungicide (C)	< 0.001	0.10		0.015	1.07		0.000	0.67	
	A X B	0.001	0.18	1.21	0.008	2.19		0.155	3.21	
	A X C	< 0.001	0.14	0.98	0.005	1.51		< 0.001	0.94	
	B X C	< 0.001	0.15	1.02	0.127	0.87		0.022	0.90	
	A X B X C	0.536	0.21		0.001	1.23	12.86	0.002	1.27	13.25

The data reported in the table refer to the level of significance (P) and the standard error of mean (sem). When interactions between factors are significant, the Fisher’s Least Significant Difference (LSD), protected by Bonferroni at $P \leq 0.05$, is reported.

Table. 4

Effect of tillage, variety susceptibility to FHB and triazole fungicide application on FHB severity of winter wheat (%); field experiments conducted at three sites in North Italy over a three years period.

			2006			2007			2008		
			Variety			Variety			Variety		
Site	Tillage	Fungicide	S	MR	Mean	S	MR	Mean	S	MR	Mean
IM	Direct sowing	Untreated	3.51	0.81	2.16	0.80	0.23	0.52	5.55	4.21	4.88
		Fungicide	0.95	0.17	0.56	0.35	0.08	0.22	1.70	0.81	1.26
		Mean	2.23	0.49	1.36	0.58	0.16	0.37	3.63	2.51	3.07
	Ploughing	Untreated	0.31	0.21	0.26	0.17	0.06	0.11	2.73	1.97	2.35
		Fungicide	0.21	0.15	0.18	0.03	0.11	0.07	0.99	0.61	0.80
		Mean	0.26	0.18	0.22	0.10	0.09	0.09	1.86	1.29	1.58
	Tillage mean	Untreated	1.91	0.51	1.21	0.48	0.15	0.31	4.14	3.09	3.62
		Fungicide	0.58	0.16	0.37	0.19	0.10	0.14	1.35	0.71	1.03
		Mean	1.25	0.34		0.34	0.12		2.74	1.90	
RC	Direct sowing	Untreated	10.59	2.28	6.43	14.91	14.38	14.65	55.72	20.09	37.91
		Fungicide	3.35	0.73	2.04	4.36	2.55	3.46	9.39	3.89	6.64
		Mean	6.97	1.51	4.24	9.64	8.47	9.05	32.55	11.99	22.27
	Ploughing	Untreated	9.38	1.53	5.45	13.05	10.28	11.66	34.72	8.66	21.69
		Fungicide	2.12	0.14	1.13	4.44	2.01	3.23	8.07	3.14	5.61
		Mean	5.75	0.84	3.29	8.75	6.14	7.44	21.39	5.90	13.65
	Tillage mean	Untreated	9.98	1.91	5.94	13.98	12.33	13.16	45.22	14.38	29.80
		Fungicide	2.74	0.44	1.59	4.40	2.28	3.34	8.73	3.52	6.12
		Mean	6.36	1.17		9.19	7.31		26.97	8.95	
SL	Direct sowing	Untreated	7.85	3.63	5.74	26.55	7.85	17.20	30.71	37.96	34.34
		Fungicide	3.21	0.77	1.99	14.73	5.31	10.02	13.72	11.00	12.36
		Mean	5.53	2.20	3.87	20.64	6.58	13.61	22.22	24.48	23.35
	Ploughing	Untreated	4.23	1.75	2.99	10.95	6.44	8.70	13.84	8.76	11.30
		Fungicide	1.34	0.43	0.89	6.91	4.55	5.73	5.32	3.69	4.51
		Mean	2.79	1.09	1.94	8.93	5.50	7.22	9.58	6.22	7.90
	Tillage mean	Untreated	6.04	2.69	4.37	18.75	7.15	12.95	22.28	23.36	22.82
		Fungicide	2.27	0.60	1.44	10.82	4.93	7.87	9.52	7.35	8.43
		Mean	4.16	1.65		14.79	6.04		15.90	15.35	

Significance for the differences of the compared means are reported in Table 3.

FHB severity was calculated as the percentage of spikelets per ear with symptoms of disease at the soft dough stages (GS 85).

Tillage: the previous crop was grain sorghum at site IM (years 2006 and 2007) and grain maize at site IM (2008), RC and SL.

Variety: S = susceptible to FHB, MR = moderately resistant to FHB

Fungicide: metconazole was applied at 0.06 kg active ingredient (AI) ha^{-1} at wheat heading.

Table. 5

Analysis of variance for grain yield, field experiments conducted at three sites in North Italy over a three years period.

Site	Source of variation	2006 P	2006 sem	2006 protected LSD	2007 P	2007 sem	2007 protected LSD	2008 P	2008 sem	2008 protected LSD
IM	Tillage (A)	0.062	0.32		0.160	0.20		0.740	0.12	
	Variety (B)	0.039	0.26		0.001	0.22		0.566	0.12	
	Fungicide (C)	0.002	0.19		0.006	0.23		0.003	0.22	
	A X B	0.583	0.37		0.514	0.31		0.621	0.16	
	A X C	0.354	0.27		0.667	0.32		0.234	0.31	
	B X C	0.143	0.38		0.380	0.53		0.204	0.26	
	A X B X C	0.428	0.53		0.864	0.76		0.083	0.37	
RC	Tillage (A)	0.003	0.17		0.004	0.19		< 0.001	0.10	
	Variety (B)	0.001	0.26		0.002	0.15		0.282	0.33	
	Fungicide (C)	0.009	0.13		0.043	0.17		0.000	0.25	
	A X B	0.123	0.37		0.452	0.21		0.160	0.47	
	A X C	0.883	0.18		0.786	0.24		0.192	0.36	
	B X C	0.710	0.23		0.028	0.19	0.53	0.168	0.29	
	A X B X C	0.457	0.32		0.986	0.27		0.980	0.41	
SL	Tillage (A)	0.354	0.25		0.024	0.12		< 0.001	0.05	
	Variety (B)	< 0.001	0.11		< 0.001	0.08		0.289	0.09	
	Fungicide (C)	0.011	0.12		0.003	0.18		0.004	0.15	
	A X B	0.048	0.16		0.002	0.12	0.80	0.068	0.12	
	A X C	0.033	0.16		0.664	0.26		0.658	0.21	
	B X C	0.194	0.12		0.124	0.21		0.634	0.21	
	A X B X C	0.038	0.17	1.56	0.153	0.30		0.447	0.29	

The data reported in the table refer to the level of significance (P) and the standard error of mean (sem). When interactions between factors are significant, the Fisher's Least Significant Difference (LSD), protected by Bonferroni at $P \leq 0.05$, is reported.

Table. 6

Effect of tillage, variety susceptibility to FHB and triazole fungicide application on grain yield of winter wheat (t ha^{-1}); field experiments conducted at three sites in North Italy over a three years period.

			2006			2007			2008		
			Variety			Variety			Variety		
Site	Tillage	Fungicide	S	MR	Mean	S	MR	Mean	S	MR	Mean
IM	Direct sowing	Untreated	6.77	7.62	7.20	6.65	8.34	7.49	6.90	7.50	7.20
		Fungicide	7.81	8.30	8.06	7.78	9.03	8.40	8.19	7.79	7.99
		Mean	7.29	7.96	7.63	7.21	8.68	7.95	7.55	7.64	7.59
	Ploughing	Untreated	7.81	8.77	8.29	6.36	7.91	7.14	7.08	6.98	7.03
		Fungicide	9.46	9.40	9.43	7.43	8.36	7.90	8.20	8.31	8.25
		Mean	8.64	9.09	8.86	6.89	8.14	7.52	7.64	7.64	7.64
	Tillage mean	Untreated	7.29	8.20	7.75	6.50	8.12	7.31	6.99	7.24	7.11
		Fungicide	8.64	8.85	8.74	7.60	8.70	8.15	8.19	8.05	8.12
		Mean	7.96	8.52	8.24	7.05	8.41		7.59	7.64	
RC	Direct sowing	Untreated	6.24	7.26	6.75	4.92	4.61	4.77	1.86	2.59	2.23
		Fungicide	6.42	7.66	7.04	5.42	4.67	5.05	4.11	4.46	4.29
		Mean	6.33	7.46	6.89	5.17	4.64	4.91	2.99	3.53	3.26
	Ploughing	Untreated	7.63	8.25	7.94	6.15	5.98	6.07	4.40	4.50	4.45
		Fungicide	7.94	8.48	8.21	6.60	5.99	6.29	6.21	5.95	6.08
		Mean	7.78	8.37	8.08	6.38	5.99	6.18	5.31	5.23	5.27
	Tillage mean	Untreated	6.93	7.76	7.34	5.54	5.30	5.42	3.13	3.54	3.34
		Fungicide	7.18	8.07	7.62	6.01	5.33	5.67	5.16	5.21	5.18
		Mean	7.06	7.91		5.77	5.31		4.15	4.38	
SL	Direct sowing	Untreated	5.87	6.49	6.18	4.12	6.04	5.08	3.69	3.75	3.72
		Fungicide	6.22	7.39	6.80	5.44	6.58	6.01	4.41	4.19	4.30
		Mean	6.05	6.94	6.49	4.78	6.31	5.55	4.05	3.97	4.01
	Ploughing	Untreated	6.03	7.46	6.74	5.57	6.25	5.91	6.33	6.53	6.43
		Fungicide	6.22	7.48	6.85	6.39	7.04	6.71	6.98	7.24	7.11
		Mean	6.12	7.47	6.80	5.98	6.64	6.31	6.66	6.88	6.77
	Tillage mean	Untreated	5.95	6.98	6.46	4.84	6.15	5.49	5.01	5.14	5.07
		Fungicide	6.22	7.43	6.83	5.92	6.81	6.36	5.70	5.72	5.71
		Mean	6.08	7.20		5.38	6.48		5.35	5.43	

Significance for the differences of the compared means are reported in Table 5.

Tillage: the previous crop was grain sorghum at site IM (years 2006 and 2007) and grain maize at site IM (2008), RC and SL.

Variety: S = susceptible to FHB, MR = moderately resistant to FHB

Fungicide: metconazole was applied at 0.06 kg active ingredient (AI) ha^{-1} at wheat heading.

Table. 7

Analysis of variance for DON content, field experiments conducted at three sites in North Italy over a three years period.

Site	Source of variation	2006 P	2006 sem	2006 protected LSD	2007 P	2007 sem	2007 protected LSD	2008 P	2008 sem	2008 protected LSD
IM	Tillage (A)	0.004	56		0.043	23		0.015	54	
	Variety (B)	0.001	112		0.001	19		< 0.001	20	
	Fungicide (C)	0.028	47		0.004	28		0.001	38	
	A X B	0.002	159	1091	0.013	27	184	< 0.001	28	
	A X C	0.091	66		0.028	39	218	0.001	54	
	B X C	0.108	19		0.016	39	219	0.003	38	
	A X B X C	0.521	27		0.204	55		0.005	54	569
RC	Tillage (A)	< 0.001	39		0.001	5		< 0.001	1359	
	Variety (B)	< 0.001	56		0.008	42		< 0.001	1001	
	Fungicide (C)	< 0.001	67		0.001	16		< 0.001	822	
	A X B	< 0.001	79	434	0.091	60		0.428	1416	
	A X C	0.054	95		0.028	22		< 0.001	1163	6354
	B X C	< 0.001	97	528	0.029	22		< 0.001	623	3404
	A X B X C	0.838	137		0.032	32	238	0.819	881	
SL	Tillage (A)	0.018	5		< 0.001	5		< 0.001	120	
	Variety (B)	< 0.001	7		0.001	65		0.001	489	
	Fungicide (C)	0.001	5		0.001	36		0.005	1262	
	A X B	0.002	9		0.044	91	626	0.359	691	
	A X C	0.003	7		0.018	51	352	0.082	1784	
	B X C	0.001	7		0.601	150		0.002	125	
	A X B X C	0.003	11	110	0.606	212		< 0.001	176	1845

The data reported in the table refer to the level of significance (P) and the standard error of mean (sem). When interactions between factors are significant, the Fisher's Least Significant Difference (LSD), protected by Bonferroni at P ≤ 0.05, is reported.

Table. 8

Effect of tillage, variety susceptibility to FHB and triazole fungicide application on DON contamination of winter wheat (μg kg^{-1}); field experiments conducted at three sites in North Italy over a three years period.

			2006			2007			2008		
			Variety			Variety			Variety		
Site	Tillage	Fungicide	S	MR	Mean	S	MR	Mean	S	MR	Mean
IM	Direct sowing	Untreated	1708	407	1057	374	96	235	1073	369	721
		Fungicide	1508	233	871	85	30	57	364	113	238
		Mean	1608	320	964	230	63	146	718	241	480
	Ploughing	Untreated	181	37	109	115	n.d.	63	86	37	62
		Fungicide	116	24	70	n.d.	n.d.	10	38	n.d.	24
		Mean	149	31	90	63	n.d.	36	62	24	43
	Tillage mean	Untreated	945	222	583	245	53	149	580	203	391
		Fungicide	812	129	471	47	20	34	201	61	131
		Mean	878	175		146	37		390	132	
RC	Direct sowing	Untreated	2552	211	1381	261	66	163	27223	22338	24781
		Fungicide	1113	27	570	130	38	84	14392	12294	13343
		Mean	1832	119	976	195	52	124	20808	17316	19062
	Ploughing	Untreated	1491	21	756	93	47	70	12951	7206	10078
		Fungicide	255	n.d.	132	64	20	42	5379	2177	3778
		Mean	873	15	444	78	33	56	9165	4691	6928
	Tillage mean	Untreated	2021	116	1069	177	56	117	20087	14772	17429
		Fungicide	684	18	351	97	29	63	9886	7235	8561
		Mean	1353	67		137	43		14986	11004	
SL	Direct sowing	Untreated	167	n.d.	88	1103	547	825	19804	18163	18983
		Fungicide	47	n.d.	28	768	213	490	14885	9135	12010
		Mean	107	n.d.	58	935	380	658	17345	13649	15497
	Ploughing	Untreated	50	n.d.	30	446	71	258	6513	2440	4477
		Fungicide	29	n.d.	20	225	18	122	2560	673	1616
		Mean	40	n.d.	25	335	45	190	4537	1556	3046
	Tillage mean	Untreated	108	n.d.	59	774	309	542	13159	10301	11730
		Fungicide	38	n.d.	24	496	116	306	8723	4904	6813
		Mean	73	n.d.		635	212		10941	7603	

Significance for the differences of the compared means are reported in Table 7.

Tillage: the previous crop was grain sorghum at site IM (years 2006 and 2007) and grain maize at site IM (2008), RC and SL.

Variety: S = susceptible to FHB, MR = moderately resistant to FHB

Fungicide: metconazole was applied at 0.06 kg active ingredient (AI) ha^{-1} at wheat heading.

nd: not detected. The detection limit was 20 μg kg^{-1}.

Table. 9

Severity of the effect of several agricultural practices on deoxynivalenol (DON) contamination in winter wheat grain in natural conditions and on average of the other experimental factors included.

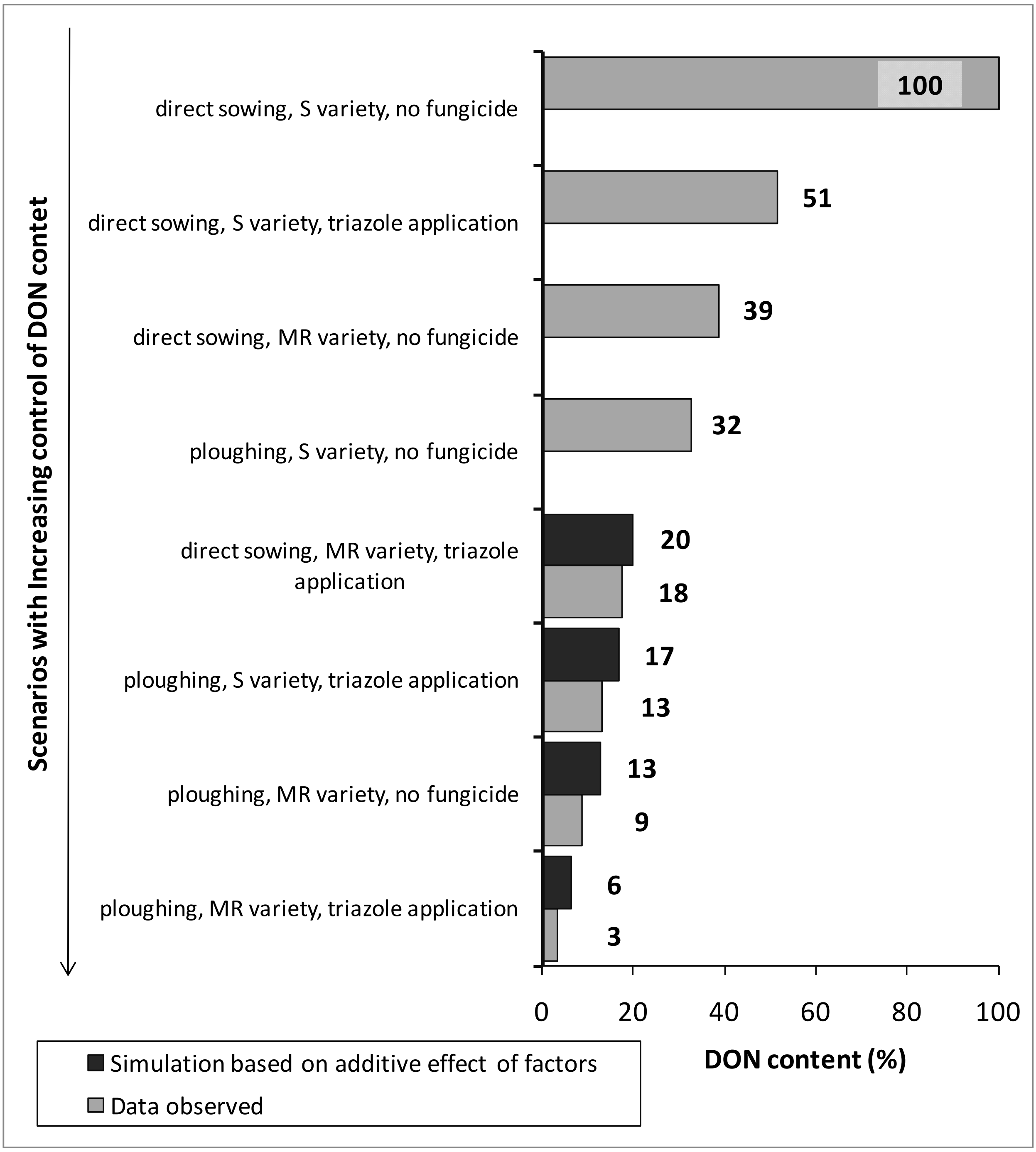
Country	Factor	Value in			Reference
		Numerator	Denominator	Severity of effect	
Italy	Tillage (after maize or sorghum)	Direct sowing	Ploughing (30 cm)	4.8	Data reported in the present manuscript
	Variety	Highly susceptible	Moderately resistant	5.5	
	Fungicide application	Without	With	2.4	
USA (MN)	Preceding crop	Maize	Soybean	2.0	Dill-Macky and Jones, 2000
	Tillage	Direct sowing	Ploughing (30 cm)	1.4	
Canada (ON)[a]	Preceding crop	Maize	Soybean	2.9	Schaafsma et al., 2001
	Tillage[b]	Minimum tillage	Ploughing (30 cm)	1.6	
	Variety	Highly susceptible	Moderately resistant	3.7	
Germany[a]	Preceding crop	Winter wheat	Sugar beet	4.3	Koch et al., 2006
	Tillage[b]	Direct sowing	Ploughing (30 cm)	2.7	
	Variety	Highly susceptible	Moderately resistant	4.3	
Germany	Tillage (after wheat)	Direct sowing	Ploughing (30 cm)	3.4	Koch et al., 2006
	Variety	Highly susceptible	Moderately resistant	5.6	
	Fungicide application	Without	With	2.1	
Italy	Variety	Highly susceptible	Moderately resistant	1.7	Blandino et al., 2006
	Fungicide application	Without	With	2.4	
Argentina	Tillage (after maize)	Direct sowing	Ploughing (30 cm)	1.3	Lori et al., 2009
	Variety	Highly susceptible	Moderately resistant	1.7	

[a] Average data obtained from commercial wheat farm fields

[b] From commercial wheat farm fields with different previous crop

Figure 1.

Effect of different agronomic scenarios, obtained from the combination of tillage, variety susceptibility and fungicide application, on the relative DON content.



Reductions are expressed in relation to the worst case scenario (direct sowing, S variety, no fungicide = 100% DON content).

The reported data are the average of the relative DON content of 9 field experiments, expressed in relation to the worst case scenario in each trial.

The simulations of the combined effect of factors were obtained from the additive computation of the effect of a single factor in relation to the worst case scenario .