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Fusarium and mycotoxin content of harvested grain was not related to tillage intensity in Norwegian spring wheat fields

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RESEARCH ARTICLE

Abstract

To mitigate the risk of erosion and nutrient runoff, reduced tillage has become more prevalent in Norway. Within within recent decades, there have been some years with relatively high occurrence of *Fusarium* head blight and mycotoxins in Norwegian cereal grain. This is thought to have been caused by an increased inoculum potential (IP) of *Fusarium* spp. due to larger amount of crop residues remaining on the soil surface, in combination with weather conditions promoting fungal growth and infection of cereal plants. The objective of this work was to elucidate the influence of different tillage practices on the IP of *Fusarium* spp. and the subsequent *Fusarium*-infection and mycotoxin contamination of spring wheat grain at harvest. Tillage trials were conducted at two locations in southeast Norway (Solør and Toten) over three years, 2010-2012. Residues of wheat from the previous year were collected in spring. *Fusarium avenaceum* and *Fusarium graminearum* were the most common *Fusarium* species recorded on wheat straw residues. IP was calculated as the percentage of the residues infested with *Fusarium* spp. multiplied by the proportion of the soil surface covered with residues. The IP of *Fusarium* spp. was lower in ploughed plots compared to those tilled with harrowing only. Ploughing in spring resulted in a similarly low IP as autumn ploughing. In contrast, harrowing in autumn generally reduced IP more than did spring harrowing. The mycotoxin levels in the harvested wheat were generally low, except for deoxynivalenol at high levels in Solør 2011. Despite a lower IP of ploughed versus harrowed plots, this was not reflected in the content of *Fusarium* and mycotoxins in harvested grain. The *Fusarium* species that dominated in the residues examined in this study were the same as those detected in the harvested grain, supporting the finding that residues are an important source of inoculum.

Keywords: *Fusarium graminearum, Fusarium avenaceum*, straw residues

1. Introduction

Fusarium head blight (FHB) is an important fungal disease of cereals (Parry *et al.,* 1995). It can cause significant yield losses and reduced grain quality. The disease is caused by several species of the genus *Fusarium*, which are known to survive in soil and on crop residues. The *Fusarium* species population composition, and its abundance in the field, is influenced by environmental conditions (Xu *et al.,* 2008). *Fusarium* spp. produce a range of mycotoxins that may contaminate grain and be harmful to both animals and humans when consumed (Desjardins, 2006).

Soil tillage is important to loosen the soil, to prepare the seedbed, for the incorporation of plant residues, and to control weeds (Håkansson *et al.,* 1998) and plant diseases (Bockus and Shroyer, 1998). Due to increased risk of soil erosion and nutrient runoff from exposed soils, the Norwegian authorities have encouraged farmers to reduce soil tillage operations. As a result, the primary tillage in Norwegian cereal fields is now more frequently performed in spring, and reduced tillage has become more prevalent (Tørresen *et al.,* 2012). In Scandinavia, ploughless soil tillage can be successful under a wide range of soil conditions (Rasmussen, 1999). Reports from several long-term trials, examining the impact of tillage practices on grain yields in Norway, indicate only small differences in grain yields

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between reduced tillage and ploughed treatments on loam soil, but reductions in yields have been linked to reduced tillage treatments on silt, sandy loam and some clay soils (Riley, 2014; Riley *et al.,* 1994, 2005, 2009). Reduced tillage lowers labour requirements and machinery costs (Riley *et al.,* 1994), and was reported to be the most profitable practice in a German study of a crop rotation systems in wheat (Verch *et al.,* 2009). Thus, for certain soil types, and under certain crop rotation systems, reduced tillage has the potential to ensure sustainable crop production with little negative influence on grain yield.

In some recent years, a high occurrence of *Fusarium* and mycotoxins has been observed in Norwegian cereals (Norwegian Scientific Committee for Food Safety, 2013) and *Fusarium graminearum* has become a prevalent species associated with FHB (Hofgaard *et al.,* 2016a). The increase in FHB is thought to be related to enhanced inoculum potential (IP) of *Fusarium* spp. resulting from the larger amount of crop residues on the soil surface, as a result of production systems where reduced tillage and monoculture cereals have been combined with weather conditions that promote *Fusarium* growth and infection (Norwegian Scientific Committee for Food Safety, 2013). Diseases caused by residue-borne pathogens are more prevalent with increasing amounts of crop residues (Bockus and Shroyer, 1998; Dill-Macky and Jones, 2000). To minimise the risk of soil erosion and nutrient runoff, and at the same time ensure suitable grain quality, it is important to identify sustainable tillage practices for Norwegian conditions.

In areas with low inoculum pressure from surrounding fields, infected residues from the previous crop are an important source of *Fusarium* spp. inoculum (McMullen *et al.,* 2012). Disease severity and mycotoxin contamination of cereals are influenced both by the type and quantity of residues from the previous crop (Dill-Macky and Jones, 2000). The IP of *Fusarium*-infested plant residues may vary with plant species, plant tissues, the stage of residue decomposition, soil biota and climate (Champeil *et al.,* 2004a; Pereyra and Dill-Macky, 2008). Temperature and moisture influence the degradation of residues as does the activity of microorganisms (Leplat *et al.,* 2013). Establishment of fungi in plants tissues before harvest and their incorporation into the soil after harvest, is considered to give plant pathogenic fungi an advantage over other saprophytic fungi (Bruehl and Lai, 1966). *F. graminearum* has been reported to survive for longer on residues at the soil surface than on buried residues (Pereyra *et al.,* 2004). The IP of *Fusarium*-infested residues may also be reduced as the formation of ascospores and macroconidia is reduced in residues that have been buried for some time (Khonga and Sutton, 1988).

Fusarium culmorum, Fusarium avenaceum, F. graminearum, Fusarium poae, and *Fusarium sporotrichioides* are the *Fusarium* species most frequently isolated from cereal crop residues (Dill-Macky and Jones, 2000; Fernandez *et al.,* 2008; Golkari *et al.,* 2008; Hofgaard *et al.,* 2016b; Köhl *et al.,* 2007; Pereyra and Dill-Macky, 2008; Postic *et al.,* 2012). In a Canadian study, *Fusarium* spp. were isolated from more than 50% of the cereal residues collected from producers' fields (Fernandez *et al.,* 2008). In a field study performed in Minnesota, wheat residues served as a substrate for ascospore production of *F. graminearum* for more than one year, and surface residues provided a substrate for the fungus for a longer period than buried residues (Pereyra *et al.,* 2004). *F. avenaceum* infestation of residues is reported to be more stable over time compared to that of *F. graminearum* (Hogg *et al.,* 2010; Palazzini *et al.,* 2013). The *Fusarium* species are differentially influenced by environmental conditions. For example, in a study of the causal organisms of FHB of wheat in four European countries over a four-year period, *F. graminearum* was associated with humid and relatively warm conditions, whereas *F. avenaceum* and *F. culmorum* were more prevalent in cool/wet/humid conditions (Xu *et al.,* 2008).

Numerous studies have focused on the effect of tillage practice on the subsequent development of *Fusarium* and mycotoxins in cereal grains (Blandino *et al.,* 2012; Dill-Macky and Jones, 2000; Guo *et al.,* 2010; Henriksen, 1999; Kaukoranta *et al.,* 2019; Munger *et al.,* 2014), and many have concluded that ploughing is the best tillage practice to reduce the risk of *Fusarium* disease development and mycotoxin contamination in cereals. However, sometimes it is difficult to prove a direct link between tillage practice and the occurrence of mycotoxins in grains, as *Fusarium* inoculum may be dispersed aerially over large distances (Lori *et al.,* 2009; Prussin *et al.,* 2014). Furthermore, the association between tillage practice and mycotoxin content may differ between *Fusarium* species (Kaukoranta *et al.,* 2019). Some studies have shown that the development of *Fusarium* and mycotoxins in cereals is more related to the amount of residues on the soil surface than to the tillage practice (Maiorano *et al.,* 2008). Relatively few studies have focused on the effect of tillage practice on the IP of crop residues within a specific field (Dill-Macky and Jones, 2000; Hofgaard *et al.,* 2016b; Munger *et al.,* 2014). Additional studies are needed to determine the impact of various tillage practices on *Fusarium* inoculum build-up in a field.

The objective of this study was to elucidate the influence of various tillage and straw coverage treatments on the IP of *Fusarium* spp. around sowing in spring and possible associations with *Fusarium* and mycotoxin contamination in harvested grain of spring wheat in Norway.

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2. Materials and methods

Field trials

Tillage trials of spring wheat were conducted at two locations in southeast Norway (Solør and Toten) during the years 2010-2012. The wheat trial at Solør was established on a silt soil (precrop oat) and the trial at Toten was established on a loam soil (precrop wheat). Description of the various tillage treatments, soil physical conditions, yield parameters, dates of seeding, tillage and harvest operations are presented in Seehusen *et al.* (2017).

Each trial had a randomised split-plot design with two replicate blocks. The two main treatments (plot size 42×15 m) comprised I: most of the straw residues removed in autumn (only stubble left) and II: straw chopped in autumn and retained on the field. To allow correct implementation of tillage treatments, the plots were separated by borders of 6 and 8 m at Solør and Toten, respectively. Within each main treatment plot, 6×15 m split-plots were established, with five tillage practices: deep autumn ploughing (DAP, 25cm), shallow spring ploughing (SSP, 12-15 cm), deep autumn harrowing (DAH, 10 cm), shallow autumn harrowing (SAH, 5 cm), and shallow spring harrowing (SSH, 6 cm). The location of the plots was fixed throughout the experimental period (2010-2012). Due to limited resources, no plant material was collected in spring from the plots with deep autumn harrowing, thus this treatment was not included in the analyses. The proportion of the soil surface covered with straw residues was recorded within a week after sowing in each year (Seehusen *et al.,* 2017). In 2011, the average percentage of the field plot covered with straw residues varied with the different tillage practices from 2-32% (Solør) and <1-15% (Toten). In 2012, the average percentage of the field plot covered with straw residues varied from <1-25% (Solør) and <1-27% (Toten). No fungicides were applied in these trials.

Assessment of *Fusarium* in straw residues

Wheat straw was collected each year at both field locations within a week of sowing for the assessment of *Fusarium* spp. in the residues. In the first year of the experiment, residues were collected across the whole field, in order to calculate the general *Fusarium* spp. infestation. In 2011 and 2012, residues were collected from plots with the following treatments: DAP, SSP, SAH, and SSH, including both residue treatments (straw removed, or straw retained). Within each experimental plot, residues were collected from four 1×1 m quadrats outside the area to be harvested. The residues were dried at 25 °C for 24 h and stored at room temperature until used for the *Fusarium* spp. analysis.

For the recovery of *Fusarium* species, 50 pieces of straw from each plot were analysed, except for Solør in 2011, where 100 straw segments were used. The pieces were 1.5-2 cm long and most included a node. The method for identification of *Fusarium* species on straw has been previously described (Hofgaard *et al.,* 2016b). Briefly, the straw pieces were surface disinfected, transferred to a *Fusarium*-selective medium and incubated at 20 °C with a 12 h light cycle for 7-10 days. The percentage of *Fusarium*-infested straw residues was calculated as the number of straw pieces infested with *Fusarium* versus the total number of pieces analysed.

The IP (percentage) was calculated for each plot as the percentage of the straw residue infested with *Fusarium* spp. multiplied with the proportion (0-1) of the soil surface covered with residue after sowing as described previously (Hofgaard *et al.,* 2016b). The proportions of the surface of the plots covered with residue after sowing for each treatment are presented in Seehusen *et al.* 2017.

Assessment of *Fusarium* in harvested grains

After harvest, the grain samples of the spring wheat were dried to 10-14% moisture content and cleaned of impurities and dust by gentle air blowing. A subsample of about 200 g was obtained from each sample by passing the larger samples through a riffle type divider (Rationel Kornservice AS, Denmark). The subsamples were milled using a high speed rotor mill with a sieve size of 1 mm (ZM 200, Retsch, Haan, Germany), and then stored in a freezer at -20 °C until used for the assessment of *Fusarium* and mycotoxins.

The grain samples were analysed by quantitative PCR (qPCR) to determine the relative content of *F. graminearum, F. culmorum,* and *F. avenaceum* according to methods previously described (Halstensen *et al.,* 2006; Waalwijk *et al.,* 2004) and qPCR was performed as described in Hofgaard *et al.* (2016b). The fungal contents are presented as pg fungal DNA per ng of plant DNA. The DNA extraction and quantification of plant DNA is presented in a previous publication (Divon *et al.,* 2012).

Assessment of mycotoxins in harvested grains

The grain samples harvested in 2011 were analysed for the content of deoxynivalenol (DON), HT-2 toxin (HT-2) and T-2 toxin (T-2) by liquid chromatography-mass spectrometry (LC-MS/MS) according to the following procedure: A 5 g aliquot of each milled grain sample was extracted with 20 ml of a 1 + 1 mixture of acetonitrile and water. Then the content of a Supel™QuE Citrate Extraction tube was added, the sample was mixed well and centrifuged. 5 ml supernatant was frozen at -18 °C for removal of fats. After thawing, 1 g calcium chloride was added to the liquid phase, centrifuged again and then PSA/C18 was

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added for further clean-up of the acetonitrile phase. The sample was mixed, centrifuged, and 1 ml supernatant was then evaporated to near dryness. Finally, the sample was dissolved in 0.5 ml acetonitrile and analysed by LC-MS/MS.

The analyses were carried out using a Waters 2695 Separation Module (Waters Corporation, Milford, MA, USA) connected to a Waters Ultima Pt MS/MS-detector. The column was an Alltima C18 (Alltech, Deerfield, IL, USA) with dimensions of 150×2.1 mm i.d. and with a particle diameter of 5 µm. The injection volume was 5 µl, the flow 0.3 ml/min and the column temperature 30 °C. The mobile phase for HT-2/T-2 was: A – 5 mM ammonium acetate in water and B – methanol. A linear gradient was used starting with 75% A to 5% A in 7 min, held to 12 min and then to 75% A for 13 min and held to 15 min. The total run time was 15 min. The mobile phase for DON was: A – 5 mM formic acid in water and B – acetonitrile. A linear gradient was used starting with 85% A to 5% A in 7 min, held to 11 min and then to 85% A for 11.2 min and held to 15 min. The total run time was 15 min. The LC-MS/MS detector was used in the ES^+ mode for both HT-2 and T-2 and in the ES^- mode for DON. Parameters for the mass spectrometric detection of the analytes are shown in Table 1.

The grain samples harvested in 2012 were analysed for the content of thirteen different mycotoxins by LC-MS/MS. The following mycotoxins were analysed: DON, DON-3-glucoside (DON-3G), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), nivalenol (NIV), zearalenone (ZEA), enniatin A (ENN A), enniatin A1 (ENN A1), enniatin B (ENN B), enniatin B1 (ENN B1), HT-2, T-2 and beauvericin (BEA). The sample preparation was done according to a published procedure (Klötzel and Lauber 2017), except that only 5 g aliquot of each sample was extracted with 20 ml mixture of acetonitrile and water (80:20, v/v). The analyses were carried out using the same LC-MS/MS module as described above. Injection volume and column temperature were also the same.

The mobile phase for mycotoxins run in positive mode (HT-2, T-2, 15-ADON, ENN B, ENN B1, ENN A1, ENN A, and BEA) on LC-MS/MS instrument was as described above. The mobile phase for the mycotoxins run in negative mode (DON, NIV, DON-3G, 3-ADON, and ZEA) on the LC-MS/MS was: A – 5 mM ammonium acetate in water and B – acetonitrile. A linear gradient was used starting with 85% A to 5% A in 7 min, held to 12 min and then to 85% A for 13 min and held to 15 min. The total run time was 15 min. Parameters for the mass spectrometric detection of the analytes are shown in Table 2.

Weather data

Weather data (temperature and precipitation) for the two field locations were collected from the nearest weather stations provided by the Norwegian Agricultural Meteorology Service. Historical normal values at these locations (1961-1990) were provided by the Norwegian Meteorological Institute (Supplementary Table S1).

Statistical analysis

Analyses of variances were performed using the mixed effects model in Minitab® (v. 18.1; State College, PA, USA) to study whether different tillage and straw coverage regimes had any impact on the *Fusarium*-infestation levels on straw residue, the IP, or the content of *Fusarium* DNA and mycotoxins in the harvested grains. The following data were included in the analysis: the percentage residues infested with *F. avenaceum*, *F. graminearum* and *F. culmorum,* the IP per plot within each field experiment, the amount of DNA for each of the three *Fusarium* species, as well as the content of DON and ENN B + ENN B1 in harvested grain. The usual residual plots indicated no critical deviations from the assumptions of the response variables being normally distributed with homogeneous variance. Thus, no transformation was performed on the response variables. Within each field experiment, there were two plots (replicates) of each tillage-straw treatment. Data from each field and year were analysed separately. First, tillage treatment (nested within whole plots) and straw removal (whole plots, nested within blocks) were used as factors in the statistical model in which block was used as a random factor. Due to significant two-way interactions detected between tillage treatment and straw removal treatments in some fields, the combination of tillage treatment and straw removal treatment was used as a factor in the further analysis. Significant treatment effects were identified by applying Tukey pairwise comparisons at 5% probability level (Minitab).

Table 1. Parameters for the mass spectrometric detection of the analytes including retention times, cone voltage, precursor ions, product ions and collision energy (grain samples harvested 2011).

Mycotoxin	Retention time (min)	Precursor ion (*m/z*)	Cone voltage (V)	Product ions (*m/z*)	Collision energy (eV)
HT-2 toxin	8.9	442.20 $(M+NH_4)^+$	42	263.20, 215.04	16, 16
T-2 toxin	9.9	484.19 $(M+NH_4)^+$	49	305.20, 215.08	12, 16
Deoxynivalenol	2.5	341.30 $(M+HCOO)^-$	35	265.13, 138.07	12, 18

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Table 2. Parameters for the mass spectrometric detection of the analytes including retention times, cone voltage, precursor ions, product ions and collision energy (grain samples harvested 2012).

Mycotoxin[1]	Retention time (min)	Precursor ion (*m/z*)	Cone voltage (V)	Product ions (*m/z*)	Collision energy (eV)
HT-2	8.9	442.20 $(M+NH_4)^+$	42	263.20, 215.04	16, 16
T-2	9.5	484.19 $(M+NH_4)^+$	49	305.20, 215.08	12, 16
15-ADON	5.7	339.10 $(M+H)^+$	35	321.10, 261.10	6, 12
ENN B	11.6	657.50 $(M+NH_4)^+$	70	214.10, 196.10	36, 36
ENN B1	11.9	671.50 $(M+NH_4)^+$	60	214.10, 196.10	30, 30
ENN A1	12.4	685.50 $(M+NH_4)^+$	60	228.10, 210.10	30, 30
ENN A	12.1	699.50 $(M+NH_4)^+$	60	228.15, 210.12	30, 30
BEA	11.8	801.50 $(M+NH_4)^+$	70	262.10, 244.10	30, 30
DON	2.5	355.10 $(M+CH_3COO)^-$	35	295.06, 265.00	18, 24
NIV	1.5	311.16 $(M-H)^-$	35	281.17, 233.05	12, 12
D3G	1.4	517.10 $(M+CH_3COO)^-$	43	427.10, 247.13	24, 27
3-ADON	5.6	337.10 $(M-H)^-$	35	307.10, 172.90	12, 12
ZEA	9.0	317.17 $(M-H)^-$	50	175.09, 131.09	24, 24

[1] DON = deoxynivalenol; DON-3G = DON-3-glucoside; 3-ADON = 3-acetyldeoxynivalenol; 15-ADON = 15-acetyldeoxynivalenol; NIV = nivalenol; ZEA = zearalenone; ENN A enniatin A; ENN A1 = enniatin A1; ENN B = enniatin B; ENN B1 = enniatin B1; HT-2 = HT-2 toxin; T-2 = T-2 toxin; BEA = beauvericin.

Regression analyses (Minitab) were performed to identify possible associations between the percentage *F. avenaceum*-versus *F. graminearum*-infested straw, between the DNA content of *F. avenaceum* versus *F. graminearum* in the harvested grain, as well as the *Fusarium* DNA content versus mycotoxins in harvested grains.

3. Results

Fusarium on straw residues

F. avenaceum was the most prevalent *Fusarium* species isolated from straw residues of wheat at both locations over the three-year study (Figure 1). In both fields, the yearly average percentage *F. avenaceum*-infested residues increased during the experimental period, from 42 to 94% at Solør and from 89 to 98% at Toten (Figure 1). The proportion of straw residues infested with *F. avenaceum* within a single field plot varied from 58 to 100% (median 93%) across all plots analysed in 2011 and 2012. Tillage treatments in combination with straw removal treatments did not significantly influence the percentage of *F. avenaceum*-infested residues in most fields (Table 3). However, there was a tendency towards a slightly lower proportion of *F. avenaceum*-infested residues in the ploughed versus the harrowed treatments in both years, and a significant difference was observed between DAP and SSH plots in Solør 2012.

F. graminearum was the second most common *Fusarium* species isolated from straw residues in the study (Figure 1). The percentage of *F. graminearum* infestation was lower in 2012 than 2011 for both fields. The highest yearly average levels of infestation were observed at Solør, whereas at Toten the levels of *F. graminearum*-infested residues were generally low. The proportion of straw residues infested with *F. graminearum* varied from 1 to 60% across fields. Tillage treatment in combination with straw removal did not significantly influence the percentage of *F. graminearum*-infested residues (Table 3).

The average infestation of residues by *F. culmorum* was low (from 0-10%) in both fields in all years of the study. At Toten, the levels of *F. culmorum*-infested residues were similar to those of *F. graminearum*, whereas at Solør only low levels (1% or less) of *F. culmorum* were detected (Figure 1). Other *Fusarium* species detected in the residues were *Fusarium langsethiae, Fusarium tricinctum, Fusarium cerealis, F. sporotrichioides, F. poae* and *Fusarium equiseti*, but their average levels were generally below 1% (data not shown).

Inoculum potential of *Fusarium* in relation to tillage and straw management

IP was calculated as the percentage of the residues infested with *Fusarium* spp. multiplied by the proportion of the soil surface covered with residues. Tillage significantly influenced the IP of *F. avenaceum* and *F. graminearum* at both locations, whereas no significant effect of the straw removal treatment was found. As significant interactions between the straw removal and tillage treatments were found within the fields, Tukey pairwise comparisons were

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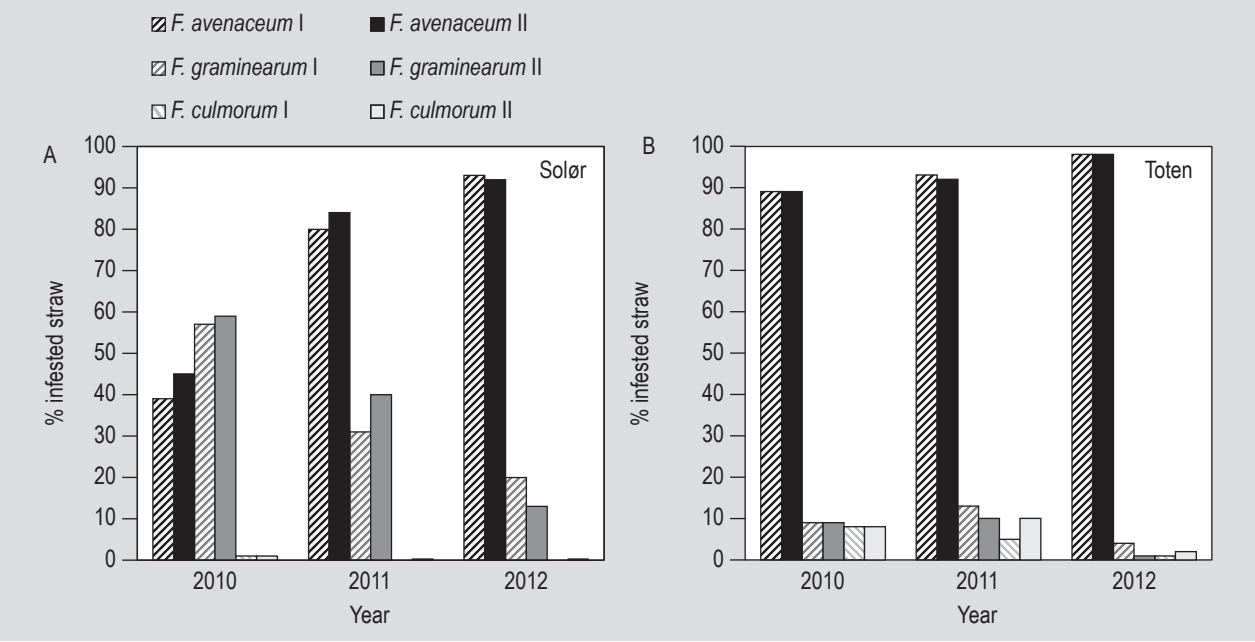

Figure 1. Percentage of *Fusarium*-infested wheat straw residues on the soil surface at sowing in field experiments at two locations over three years (2010, 2011 and 2012). (A) Solør (precrop, oats in 2009), and (B) Toten (precrop, wheat in 2009). Hatched bars represent data from plots where most of the straw was removed (I) and filled bars indicate that the straw was chopped and retained in the field (II). Three *Fusarium* species were examined: *F. avenaceum*, *F. graminearum* and *F. culmorum*.

performed to identify significant differences in the IP of *Fusarium* species between treatments within a field (straw removal treatment combined with tillage treatment).

Generally, the IPs for *F. avenaceum* were higher than those for *F. graminearum* over the two years, and the IPs were higher on harrowed compared to ploughed plots (Figure 2). Within a location, the highest IPs were found in the spring-harrowed treatments where the straw was retained after harvest. The IPs for *F. culmorum* were generally low, however significantly higher IP of *F. culmorum* was observed on spring-harrowed plots where the straw was retained after harvest compared to ploughed plots where the straw was removed in Toten in 2011 (Table 3). In fields with an IP (for any *Fusarium* species) above 10% in spring-harrowed treatments (mostly recorded for *F. avenaceum*), significant differences in IP were generally evident between harrowed and ploughed treatments. Straw removal was observed to significantly reduce the IP of *F. avenaceum* and *F. graminearum* in some of the spring-harrowed treatments where the IPs were high (Figure 2). Significant effect of tillage treatment on the IP of a *Fusarium* species was mainly detected in fields where a species was isolated from more than 20% of the residues, e.g. *F. avenaceum* at both locations and years, and *F. graminearum* at Solør in both years. No significant differences in IPs were found between spring and autumn ploughed treatments.

Fusarium and mycotoxins in harvested grains

The relative contents of *F. graminearum*, *F. culmorum* and *F. avenaceum* were analysed in the harvested grain by qPCR. *Fusarium graminearum* and *F. avenaceum* were the most commonly detected species (Table 3). The DNA levels were mostly higher for *F. graminearum* than for *F. avenaceum*, whereas the DNA-levels of *F. culmorum* were generally low (0-29 pg/µg).

The average DNA-levels of *F. avenaceum* in harvested grain ranged from 21 to 827 pg/µg for the treatments across locations and years. DNA-levels were higher in 2011 than in 2012 at both locations (Table 3). No significant differences in the DNA-levels of *F. avenaceum* were detected in grain harvested from plots receiving different treatments within a field in either year.

The enniatin-content of harvested grains were only analysed in 2012 and generally low levels were detected (Table 3). No significant differences in enniatins were detected in grain harvested among the different treatments within either field experiment.

There was no correlation between the content of *F. avenaceum* DNA in the grain harvested and the proportion of the wheat residues infested with *F. avenaceum* in spring (Figure 3A). However, grain with *F. avenaceum* DNA levels above 400 pg/µg was only found in treatments where more than 85% of the straw was infested with *F. avenaceum*. The

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Table 3. The percentage of the soil area covered with straw residues after sowing in spring, the percentage of *Fusarium*-infested residues (in spring), *Fusarium* inoculum potential (IP), and the content of *Fusarium* DNA and mycotoxins (in harvested grain) from four tillage-straw removal treatments in spring wheat field experiments at Solør and Toten over two years (2011 & 2012).

Field[1]	Treatm[2]	%RC[3]	Infested straw[4] (%)			IP[5]			Mycotoxin[6] (µg/kg)		DNA[7] (pg/µg)		
			Fa	Fg	Fc	Fa	Fg	Fc	DON	ENNs	Fa	Fg	Fc
Solør	DAP-I	2	86	35	0	1.7	0.7	0.0	2,138	-	255	3,314	2
2011	DAP-II	2	89	35	0	1.3	0.5	0.0	2,138	-	282	4,110	0
	SSP-I	4	73	36	0	2.5	1.3	0.0	1,519	-	109	2,163	0
	SSP-II	4	80	43	0	2.8	1.6	0.0	1,817	-	237	4,446	0
	SAH-I	8	72	31	0	5.9	2.3	0.0	2,029	-	332	5,795	0
	SAH-II	11	77	46	0	8.3	5.0	0.0	1,770	-	339	3,457	0
	SSH-I	21	90	21	0	18.5	4.5	0.0	1,245	-	827	1,933	0
	SSH-II	32	88	35	1	28.2	11.2	0.3	1,532	-	441	2,802	0
P-value			ns	ns	ns	0.00	0.01	ns	ns	-	ns	ns	-
Solør	DAP-I	<1	88	28	0	0.0	0.0	0.0	253	97	99	160	0
2012	DAP-II	<1	88	18	1	0.4	0.1	0.0	207	192	122	181	0
	SSP-I	<1	93	18	0	0.5	0.1	0.0	225	207	72	88	0
	SSP-II	<1	87	13	0	0.4	0.0	0.0	393	156	117	209	0
	SAH-I	8	95	25	0	7.1	1.9	0.0	263	180	144	170	0
	SAH-II	9	96	12	0	8.7	1.0	0.0	300	336	312	346	0
	SSH-I	9	96	9	0	9.1	0.9	0.0	287	302	107	274	0
	SSH-II	25	97	10	0	24.2	2.2	0.0	293	398	222	248	0
P-value			0.05	ns	ns	0.00	0.02	ns	ns	ns	ns	ns	-
Toten	DAP-I	<1	88	16	8	0.0	0.0	0.0	153	-	176	237	4
2011	DAP-II	6	93	8	7	5.6	0.5	0.4	69	-	119	177	0
	SSP-I	<1	93	12	4	0.5	0.0	0.0	115	-	155	67	8
	SSP-II	3	91	17	17	2.3	0.3	0.4	93	-	123	105	0
	SAH-I	10	95	13	5	9.5	1.3	0.5	250	-	280	243	1
	SAH-II	11	94	5	4	9.9	0.4	0.3	163	-	132	146	19
	SSH-I	15	96	12	5	13.8	1.8	0.7	181	-	312	261	0
	SSH-II	14	90	13	11	12.6	1.8	1.5	116	-	175	106	2
P-value			ns	ns	0.04	0.001	ns	0.02	ns	-	ns	ns	ns
Toten	DAP-I	<1	95	4	0	0.0	0.0	0.0	337	159	81	288	20
2012	DAP-II	<1	96	1	2	0.0	0.0	0.0	274	98	61	99	29
	SSP-I	2	97	3	2	1.5	0.0	0.0	208	134	21	147	2
	SSP-II	3	98	1	3	2.9	0.1	0.1	268	190	39	111	4
	SAH-I	12	98	1	0	11.8	0.1	0.0	339	87	57	226	1
	SAH-II	19	98	1	2	18.1	0.2	0.3	206	199	88	187	2
	SSH-I	17	100	6	1	17.0	1.0	0.2	265	120	48	310	4
	SSH-II	27	98	0	0	26.5	0.0	0.0	282	39	59	219	0
P-value			ns	ns	ns	0.00	ns	ns	ns	ns	ns	ns	ns

[1] The experimental fields of wheat were located in southeast Norway (Solør and Toten) in the years 2011 and 2012.

[2] The following tillage treatments were included: DAP = deep autumn ploughing; SSP= shallow spring ploughing; SAH= shallow autumn harrowing; SSH= shallow spring harrowing. Two straw removal treatments were included: straw was removed (I) or chopped and retained (II) in the field. Each value is the average of two replicate plots. Ns = non-significant (P>0.05) effect of treatment according to the Mixed effects model in Minitab.

[3] RC% = percentage of the soil area covered with wheat residues after sowing in spring.

[4] The percentage of straw residues infested in spring with *Fusarium avenaceum* (Fa), *Fusarium graminearum* (Fg) and *Fusarium culmorum* (Fc).

[5] Inoculum pressure (IP) was calculated as the relative soil area covered with wheat residues after sowing in spring (0-1) multiplied by the percentage of the straw residues infested with the respective *Fusarium* species.

[6] Mycotoxin content in grains harvested from plots receiving the different tillage and straw treatments. DON = deoxynivalenol, ENNs = sum of enniatin B and B1.

[7] DNA content of *Fusarium* species in grains harvested from plots receiving the different tillage-straw removal treatments. The DNA content is presented as pg DNA of the respective *Fusarium* species per µg of plant DNA.

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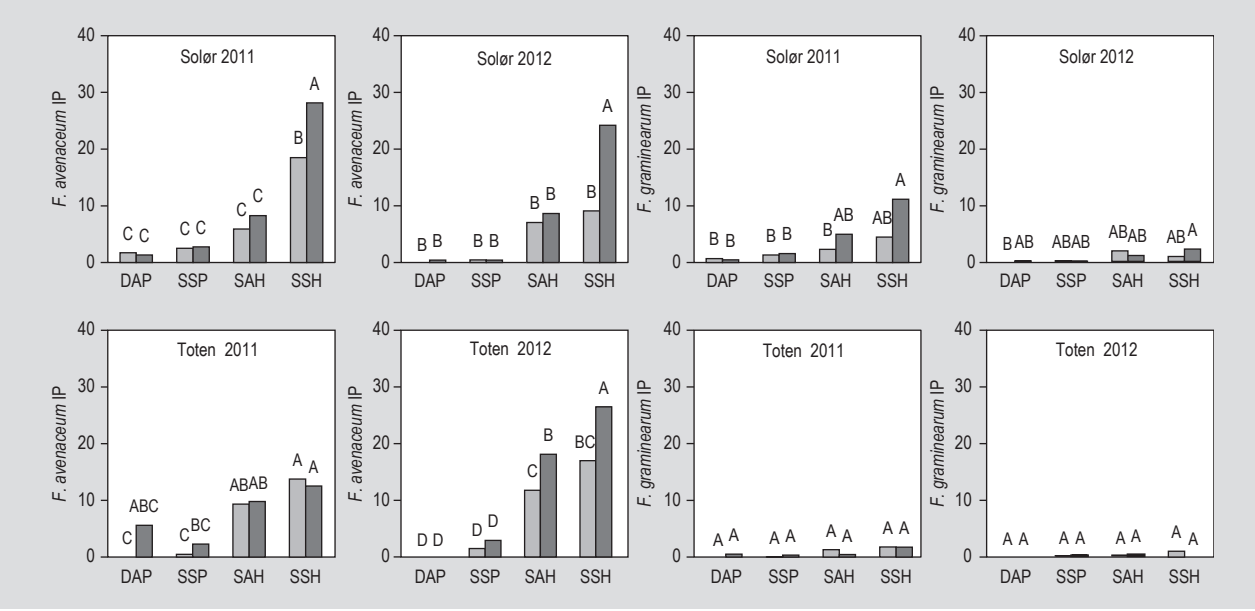

Figure 2. Effects of tillage and straw removal treatments on the inoculum potential (IP) of *Fusarium avenaceum* and *Fusarium graminearum* in field experiments conducted at two locations in southeast Norway (Solør and Toten) in 2011 and 2012. The tillage treatments: DAP = deep autumn ploughing; SSP = shallow spring ploughing; SAH = shallow autumn harrowing; SSH = shallow spring harrowing. Light bars represent data from treatments where the straw was removed; dark bars indicate the treatment where the straw was chopped and retained in the field. IP was calculated as the relative soil area covered with wheat residues after sowing in spring (0-1) multiplied by the percentage of the residues infested with the respective *Fusarium* species. Different letters over the bars indicate significant treatments effects at $P \leq 0.05$ (Tukey pairwise comparisons).

content of ENN B + ENN B1 was not significantly correlated to the DNA content of the enniatin producer *F. avenaceum* in harvested grains (Figure 3B).

The average DNA content of *F. graminearum* in the harvested grain ranged from 67 to 5,795 pg/µg across locations and years (Table 3). At Solør, the average *F. graminearum* DNA-level in harvested grain was higher in 2011 compared to 2012. Relatively low levels of *F. graminearum* DNA were observed at Toten in both years. No significant differences in *F. graminearum* DNA-levels were detected in the grain harvested from different treatments within a field in either year.

The DNA content of *F. graminearum* in harvested grain was significantly correlated to the proportion of straw residues infested with *F. graminearum* in the spring of each year of the study (P=0.000, R^2adj. = 58%, Figure 4A). As high and variable levels of *F. graminearum*-infested straw were only observed on plots at Solør location in 2011, the distribution of the percentage of *F. graminearum*-infested straw were compared with the field map. The plots which recorded the highest levels of *F. graminearum*-infested straw were situated in one part of the field, which did not correspond to the tillage or straw removal treatments (data not shown).

The DON-levels of harvested grain were generally low (below 400 µg/kg), except in Solør in 2011 where DON-levels within a plot were generally above 1000 µg/kg (Figure 4B). The DON-levels of harvested grain were significantly correlated to the DNA content of the DON-producer *F. graminearum* (P=0.000, R^2adj = 85%). Furthermore, DON levels above 1000 µg/kg were only detected in samples with a *F. graminearum*-DNA content over 1000 pg/µg. Tillage and straw removal treatment did not significantly influence the DON-levels within a field in either year of the study (Table 3).

Results from the remaining mycotoxin analyses are not presented here, as only low levels were detected (HT-2 + T-2 <23 µg/kg; DON-3G <50 µg/kg; 3-ADON <50 µg/kg; 15-ADON <50 µg/kg; ZEA <3 µg/kg; NIV <35 µg/kg; ENN A <5 µg/kg; ENN A1 <14 µg/kg; BEA <15 µg/kg) in the harvested wheat grains.

4. Discussion

The objective of this work was to elucidate the influence of different tillage and straw removal treatments on the IP of *Fusarium* spp. in Norwegian spring wheat fields and further the association between IP and *Fusarium* infestation and mycotoxin content of the harvested grain. Differences in the *Fusarium* spp. infestation of wheat straw residue were detected between the locations and years in this study. The levels of *F. avenaceum* infested straw were generally higher than those of *F. graminearum*. The amount of straw residue

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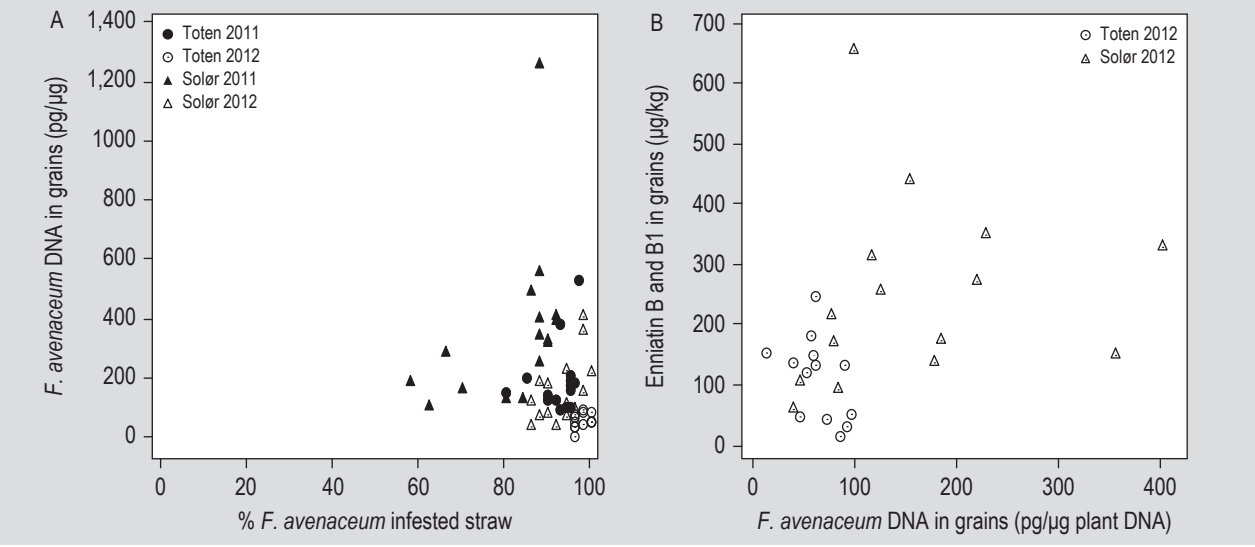

Figure 3. The content of *Fusarium avenaceum* DNA in wheat grain harvested in autumn, relative to the proportion of *Fusarium*-infested straw residues observed in spring in the respective field plot (A) and the content of enniatin B + enniatin B1 in relation to the DNA content of *F. avenaceum* in grain harvested from field plots receiving various tillage and straw removal treatments (B). Each field experiment included eight treatments (a factorial of four tillage treatments and two straw treatments). Each data point represents the results of one field plot. The DNA content is presented as pg DNA of the respective *Fusarium* species per µg plant DNA.

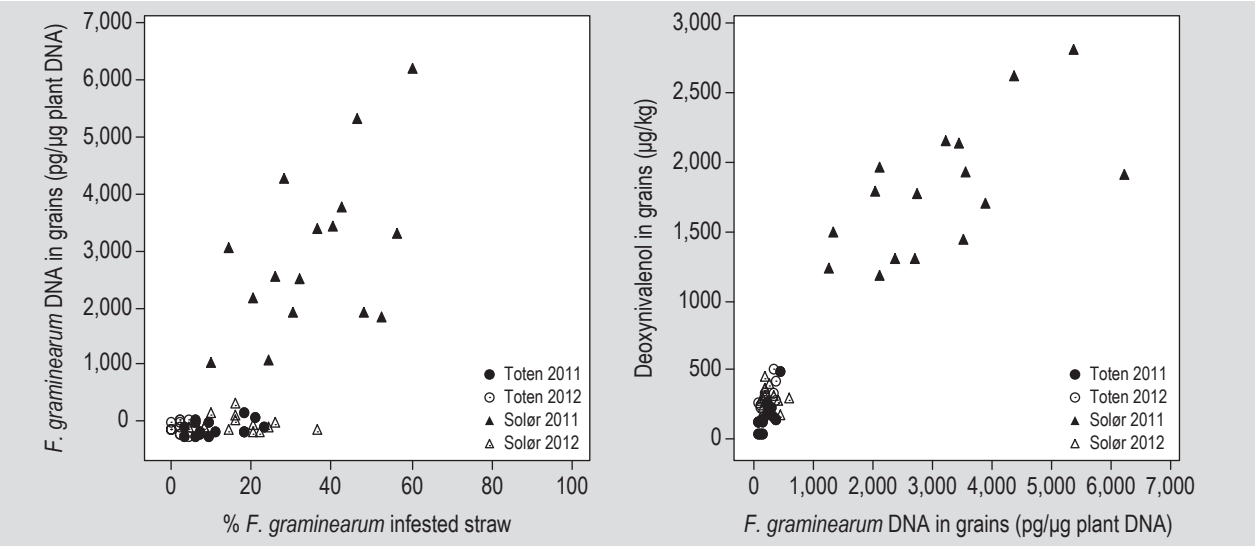

Figure 4. The content of *Fusarium graminearum*-DNA in wheat grain harvested in autumn, relative to the percentage *Fusarium*-infested straw residues observed in the spring in the respective field plot (A), and the content of deoxynivalenol, relative to *F. graminearum*-DNA, in grain harvested from field plots receiving various tillage and straw removal treatments (B). The data presented was collected from field experiments performed at two locations in Norway (Solør and Toten) over two years (2011-2012). Each field experiment included eight treatments (a factorial of four tillage treatments and two straw treatments.). Each data point represents the results of one field plot. The *F. graminearum*-DNA content is presented as pg *F. graminearum*-DNA per µg of plant DNA.

on the soil surface and thus, the IP, was lower in ploughed compared to harrowed treatments. In harrowed treatments, removal of straw residues prior to harrowing reduced the IP. Despite significant differences in IP between treatments within a field, these differences were not reflected in the contents of *Fusarium* or mycotoxins in the harvested grains.

Soil cultivation clearly influenced the *Fusarium* IP. For both *F. avenaceum* and *F. graminearum*, the lowest IPs were observed on the ploughed plots and the highest IPs on unploughed, spring harrowed plots. This is in agreement with other studies that, based on analysis of crop residues, reported reduced inoculum levels of *F. graminearum* in

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ploughed fields/plots compared to those with reduced or minimum tillage (Dill-Macky and Jones, 2000; Guo *et al.,* 2010; Hofgaard *et al.,* 2016b). In our study, the overall *Fusarium* IP within a field was related to the amount of straw, with no large differences in the percentage of *Fusarium* infested straw recorded between plots subjected to different tillage treatments. The calculated differences in *Fusarium* IP between treatments within a field were therefore mainly related to the amount of straw residues on the soil surface. This finding, that the amount of *Fusarium* spp. in wheat grain was correlated mainly with the amount of residues lying on the soil surface rather than the tillage practice is also supported by others (Maiorano *et al.,* 2008). In our study, significantly lower IPs were observed for autumn harrowed treatments compared to spring harrowed treatments within some fields, whereas the time of ploughing (autumn vs spring) did not significantly influence the IP in the ploughed plots. These differences reflect the variation in straw cover recorded for these tillage treatments as was discussed in Seehusen *et al.* (2017).

Removal of straw residues in autumn significantly reduced the IP in spring harrowed treatments in fields with high IP (above 20%). The effect of straw removal on reducing IP was also recorded in the autumn harrowed treatments, but the effect was mainly non-significant. By contrast, the removal of straw residues did not significantly influence the IP in the ploughed treatments. This different effect of straw removal in harrowed versus ploughed plots is most likely due to differences in the amount of straw left on the surface by the two tilling methods. Ploughing will reduce the amount of straw on the soil surface regardless of whether the straw is removed prior to ploughing.

Our results are in line with the general understanding of ploughing as the best means of reducing the occurrence, and thereby the IP, of *Fusarium* spp. in cereal fields (Champeil *et al.,* 2004b; Dill-Macky and Jones, 2000; Guo *et al.,* 2010; Hofgaard *et al.,* 2016b). According to our data, spring ploughing results in a similar IP as autumn ploughing. Thus, to reduce the risk of erosion and minimise *Fusarium* diseases, ploughing in spring appears to be the best option in areas where this is feasible. If harrowing is preferred, removal of cereal straw in autumn would contribute to reducing the IP of *Fusarium* spp. However, the effect of tillage on the development of FHB may not always be evident, as weather factors play an important role for fungal dispersal and infection in this disease (Lori *et al.,* 2009; Prussin *et al.,* 2014).

In our study, *F. avenaceum* was the dominant species of *Fusarium* on straw residues, although *F. graminearum* was also prevalent. These *Fusarium* species have been recorded on wheat residues elsewhere (Golkari *et al.,* 2008; Köhl *et al.,* 2007; Postic *et al.,* 2012). The *Fusarium* species detected on residues in this study were the same as those most commonly detected in Norwegian grain (Bernhoft *et al.,* 2010; Henriksen and Elen, 2005; Hofgaard *et al.,* 2016a; Kosiak *et al.,* 2003; Norwegian Scientific Committee for Food Safety, 2013). Only a few residues were infested with *F. culmorum* in our study. A similar relative ranking of the prevalent *Fusarium* species was also reported in recent studies of Norwegian wheat grain (Bernhoft *et al.,* 2010; Hofgaard *et al.,* 2016a). *Fusarium avenaceum* and *F. graminearum* were also the most dominant *Fusarium* species recorded on the stubble of oats and wheat in a Canadian study (Golkari *et al.,* 2008).

The general increase in *F. avenaceum* infestation of crop residues in our fields over the project period is in agreement with other reports of increases over time in *F. avenaceum* infestation of crop residues in monoculture cereals (Fernandez *et al.,* 2008). We also observed a yearly increase in the relative prevalence of *F. avenaceum* over *F. graminearum* on cereal residues. The infestation of wheat residues by *F. avenaceum* is reported to be more stable over time compared to infestation by *F. graminearum* (Hogg *et al.,* 2010; Köhl *et al.,* 2007; Palazzini *et al.,* 2013). Infestation of crop residues by plant pathogens may be related to differences in their ability to establish in the plant tissues before residues are incorporated into the soil (Bruehl and Lai, 1966; Hogg *et al.,* 2010; Köhl *et al.,* 2007). The difference in the relative prevalence of *Fusarium* species on crop residues in spring is related to the establishment of the fungi, as pathogens or saprophytes, and is dependent upon the prevailing weather conditions, especially in the period from flowering until harvest in the previous year (McMullen *et al.,* 2012). In our study, a reduction in the infestation of residues by *F. graminearum* was observed from 2010 to 2012. *Fusarium graminearum* has a higher *in vitro* growth rate than *F. avenaceum*, especially in the temperature range 15-30 °C (Brennan *et al.,* 2003). The monthly average temperature during cereal flowering (July) was reduced between 2010-2012 from 17.4 to 14.8 °C in Solør and from 16.3 to 14.5 °C in Toten, which is in the lower part of the temperature range examined in the study by Brennan *et al.* (2003). The significant reduction in the percentage of *F. graminearum* infested straw in spring 2012 versus 2011, may be the result of the weather conditions during flowering the previous year which was less conductive for *F. graminearum* infection.

The significant reduction in the percentage of *F. graminearum* infested straw of wheat at both locations from 2011 and 2012 may also be explained by the weather conditions in the autumn after harvest until sowing in spring the following year which may have differentially influenced the survival of this fungal species between years. The competition between *Fusarium* and other microorganisms is influenced by environmental conditions, such as temperature and moisture (El-Naggar *et al.,* 2003; Lakhesar *et al.,* 2010; Leplat *et al.,* 2013). A sharp reduction in *Fusarium*

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infestation on cereal residues has been observed in spring, and survival of *F. graminearum* seems inversely related to the decomposition rate (Köhl *et al.,* 2007; Pereyra *et al.,* 2004). Recovery and sporulation of plant pathogens on residues have been reported to decrease in warm and wet conditions, probably due to an increased decomposition rate combined with increased antagonistic activity (Lakhesar *et al.,* 2010; Zhang and Pfender, 1992). In our study, the weather at Solør and Toten in September 2011 was warmer (11.6 °C, 11.5 °C, respectively) and wetter (119 and 109 mm, respectively) compared with the previous year where the average temperatures were 9.6 and 9.2 °C, respectively and the rainfall 44 and 80 mm, respectively. In our study, the precipitation before sowing (April) at Solør was higher in 2012 than in 2011 and this may also have promoted the activity of antagonistic microorganisms. The relatively warm and moist conditions during the autumn of 2011 and the moist conditions in the spring of 2012 may have contributed to both increased degradation of plant residues and microbial competition, thereby reducing the survival of *F. graminearum* in residues. We speculate whether *F. avenaceum* has a better ability than *F. graminearum* to withstand microbial competition and survive saprophytically over a wider range of conditions. To examine this further, a comparison of *Fusarium* infested residues in autumn versus spring would merit examination.

Generally, both low levels of *F. graminearum* infested residues and low DON levels in harvested grains were detected at Toten during the experimental period. Likewise, generally low levels of DON, a toxin produced by this fungal species, has been reported in previous studies of wheat at this location (Langseth *et al.,* 1995). Both mycelial growth and production of perithecia in *F. graminearum* are strongly influenced by temperature and moisture (Brennan *et al.,* 2003; Dufault *et al.,* 2006). Reductions in perithecia production, and thereby the ability for long distance dispersal, is reported at temperatures below 16 °C (Dufault *et al.,* 2006). The average monthly temperatures at Toten during June and July in the period 1961-1990 have been 13.7 and 14.8 °C, which is 0.5 °C below the normal values calculated at the other location (Solør) included in this study. We speculate that the lower occurrence of *F. graminearum* at Toten may be due to the lower temperatures during heading and flowering of cereals at this location, which appear to be suboptimal for the growth, dispersal and infection of cereals by *F. graminearum*. Another relevant factor is that the field at Toten was established on a loam soil, a soil type which has better drainage, and thus greater water and air permeability, than silty and clay soil types (Riley, 1996), thus often resulting in lower moisture levels at the soil surface. In line with this, higher air permeability and a higher relative distribution of large soil pores >30 µm was measured in our field at Toten versus Solør (Seehusen *et al.,* 2017). In addition, slightly lower normal values of precipitation were recorded in the period prior to cereal flowering (May-June) at Toten, compared to the site at Solør, which may have contributed to a reduced development of *F. graminearum* at Toten. In 2009 and 2010, both temperature and precipitation were higher than normal during the flowering period for cereals, this would have favoured FHB and may explain why *F. graminearum* was recorded in the residues at Toten in 2010 and 2011. The relative abundance of *F. avenaceum,* in comparison to *F. graminearum,* at Toten may most likely be explained by the different temperature and moisture preferences of these two species (Xu *et al.,* 2008).

No significant differences in mycotoxin or *Fusarium* DNA content of harvested grains were detected in the wheat receiving different tillage and straw removal treatments within a field, despite significant differences in IPs that were detected among the treatments established in this study. This is in contrast to other studies where relationship between cropping practices, inoculum levels and development of *Fusarium* in monoculture cereals have been reported (Dill-Macky and Jones, 2000; Guo *et al.,* 2010; Henriksen, 1999). However, the effect of ploughing as a means of reducing the inoculum pressure of *Fusarium* spp. is not always clear (Miller *et al.,* 1998; Munger *et al.,* 2014). The poor relationship between IP and *Fusarium*-mycotoxin contamination of grain in our study may be a result of the generally low percentage of the soil area covered with wheat residues in spring (from <1 to 32% across fields and treatments), and aligns with other studies where only slight differences in FHB development were reported between tillage treatment where the residue cover was below 30% (Koch *et al.,* 2006). In studies where the residue cover is high, as with maize as a precrop, a reduction of mycotoxin contamination by ploughing is more likely (Blandino *et al.,* 2012).

Generally, the mycotoxin levels were low in our study, except for Solør in 2011 where high levels of DON (above 1000 µg/kg) and *F. graminearum* DNA were detected in the grain at harvest. These levels were significantly correlated with the percentage of *F. graminearum* infested debris observed within the respective plots in the spring. The differences in percentage of infested debris between plots within this field seemed related to factors other than tillage or straw removal. Within-field variation in DON content has been associated with variations in soil texture, drainage conditions and crop density (Söderström and Börjesson, 2013).

In conclusion, our results are in line with the general understanding of ploughing as a means of reducing the IP of *Fusarium* spp. However, tillage practice or straw removal did not significantly influence the *Fusarium* and mycotoxins in the grains harvested in our study. This may be a result of the generally low *Fusarium* and mycotoxin levels as well as a within-field variation in *Fusarium* and mycotoxin content influenced by factors other than the

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experimental treatments. The *Fusarium* species that dominated on straw residues in this study were the same as those most commonly detected in Norwegian grain, with the infestation levels of residues by *F. graminearum* being generally lower than for *F. avenaceum.* The DNA content of *F. graminearum* in harvested wheat grain was positively associated with the percentage of *F. graminearum* infested straw residues in spring, which supports the findings of previous studies that residues are an important source of *Fusarium* inoculum.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/WMJ2020.2575.

Table S1. Monthly average temperature, precipitation and historical normal values in the period 1961-1990, at two locations (Solør and Toten) in southeast Norway during the four-year period 2009 to 2012.

Conflict of interest

The authors declare no conflict of interest.

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