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Agricultural factors affecting *Fusarium* communities in wheat kernels

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease of cereals caused by *Fusarium* fungi. The disease is of great economic importance especially owing to reduced grain quality due to contamination by a range of mycotoxins produced by *Fusarium*. Disease control and prediction is difficult because of the many *Fusarium* species associated with FHB. Different species may respond differently to control methods and can have both competitive and synergistic interactions. Therefore, it is important to understand how agricultural practices affect *Fusarium* at the community level.

Lower levels of *Fusarium* mycotoxin contamination of organically produced cereals compared with conventionally produced have been reported, but the causes of these differences are not well understood. The aim of our study was to investigate the effect of agricultural factors on *Fusarium* abundance and community composition in different cropping systems. Winter wheat kernels were collected from 18 organically and conventionally cultivated fields in Sweden, paired based on their geographical distance and the wheat cultivar grown. We characterised the *Fusarium* community in harvested wheat kernels using 454 sequencing of translation elongation factor 1-α amplicons. In addition, we quantified *Fusarium* spp. using real-time PCR to reveal differences in biomass between fields.

We identified 12 *Fusarium* operational taxonomic units (OTUs) with a median of 4.5 OTUs per field. *Fusarium graminearum* was the most abundant species, while *F. avenaceum* had the highest occurrence. The abundance of *Fusarium* spp. ranged two orders of magnitude between fields. Two pairs of *Fusarium* species co-occurred between fields: *F. poae* with *F. tricinctum* and *F. culmorum* with *F. sporotrichoides*. We could not detect any difference in *Fusarium* communities between the organic and conventional systems. However, agricultural intensity, measured as the number of pesticide applications and the amount of nitrogen fertiliser applied, had an impact on *Fusarium* communities, specifically increasing the abundance of *F. tricinctum*. There were geographical differences in the *Fusarium* community composition where *F. graminearum* was more abundant in the western part of Sweden. The application of amplicon sequencing provided a comprehensive view of the *Fusarium* community in cereals. This gives us better opportunities to understand the ecology of *Fusarium* spp., which is important in order to limit FHB and mycotoxin contamination in cereals.

1. Introduction

Fusarium head blight (FHB) is an economically important disease in cereal production world-wide caused by a range of *Fusarium* species. The disease can cause important yield losses, but the most problematic aspect of FHB is the associated contamination by harmful mycotoxins produced by many *Fusarium* species (Parry et al., 1995). Important *Fusarium* toxins include deoxynivalenol (DON), nivalenol (NIV), HT-2 and T-2, zearalenone (ZEA) and fumonisins, that have negative effects on human and animal health (D'Mello et al., 1999; Reddy et al., 2010). Furthermore, the possible health effects of several more recently discovered *Fusarium* toxins are not well understood (Jestoi, 2008). In the EU, there are legislative limits for DON, ZEA and fumonisins in cereals designated for human consumption and recommended limits for animal feed (European Commission, 2006a, 2006b). It is important to reduce human and animal exposure to *Fusarium* mycotoxins as well as the economic consequences for the farmer. These can be considerable since contaminated grain cannot be sold for food or feed. *Fusarium* fungi can produce significant amounts of mycotoxins already when the crop is growing in the field. Therefore, it is crucial to develop control measures that can reduce *Fusarium* infection and mycotoxin contamination in the field.

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Fusarium head blight is caused by several *Fusarium* species of which *F. graminearum*, *F. culmorum* and *F. avenaceum* predominate, but up to 17 species have been associated with the disease (Parry et al., 1995). The many species involved in FHB makes the disease difficult to control. Different *Fusarium* species have different life cycles, host range, mycotoxin profiles, climatic preferences and respond differently to control methods (Xu and Nicholson, 2009). A better understanding of how environmental and agronomic factors affect the *Fusarium* community is important in order to make predictions and develop disease control strategies.

The *Fusarium* species involved in FHB generally produce asexual conidia, but some have sexual stages producing ascospores, e.g. *F. graminearum* (teleomorph *Gibberella zeae*) and *F. avenaceum* (teleomorph *G. avenacea*). Others, e.g. *F. culmorum* produce thick-walled chlamydospores that can persist for years in soil (Sitton and Cook, 1981). *Fusarium* survives saprotrophically on crop residues in the field and can infect the following crop (Champeil et al., 2004; Parry et al., 1995). Dispersal of conidia from crop residues by rain splashing has been proposed as a major infection route, but wind dispersal can be important, especially for species producing ascospores (Champeil et al., 2004; Keller et al., 2014).

Several agricultural practices and environmental factors are known to increase the risk of FHB (Champeil et al., 2004; Edwards, 2004). The crop is most susceptible at flowering and warm and wet weather conditions during this period favour *F. graminearum* infection and subsequent DON contamination (Obst et al., 1997). High nitrogen application and minimum tillage appears to be risk factors of *Fusarium* infection (Martin, 1991; Obst et al., 1997). The preceding crop is also important, where maize has been associated with the highest risk (Dill-Macky and Jones, 2000; Obst et al., 1997).

Fungicides have often shown limited and inconsistent efficacy against *Fusarium* infection and mycotoxin contamination (Parry et al., 1995). For example, in field trials inoculated with *F. culmorum* or *F. graminearum* FHB disease severity was reduced by 25–77% depending on cultivar, location and type of fungicide (Haidukowski et al., 2005). Fungicide application at flowering appears to be the most efficient in reducing *Fusarium* incidence and DON contamination (Chala et al., 2003). In contrast, fungicide application at the beginning of heading to target leaf diseases may even increase *Fusarium* infection (Henriksen and Elen, 2005).

Interestingly, several studies have shown lower or equal levels of *Fusarium* and mycotoxin contamination in organic cereal production than in conventional production (Bernhoft et al., 2010; Birzele et al., 2002; Edwards, 2009; Gottschalk et al., 2009; Meister, 2009). In the UK, Edwards (2009) observed lower levels of HT-2 and T-2 in organically produced wheat, but no difference in DON contamination. Other studies identified lower DON levels in organically produced wheat than in conventionally produced (Bernhoft et al., 2010; Birzele et al., 2002). The cause of the lower mycotoxin contamination in organic production is poorly understood, but certain farming practices differing between the two systems have been proposed as explanations (Bernhoft et al., 2010; Edwards, 2009).

Characterising *Fusarium* communities using culturing is time-consuming and risk overestimating fast-growing species. Real-time PCR detection with species-specific primers requires many assays to cover all species involved in FHB. High-throughput amplicon sequencing (HTS) has enabled larger and more detailed studies of fungal communities (Meiser et al., 2014). However, the internal transcribed spacer (ITS), which is commonly used as a barcode for fungi (Schoch et al., 2012), lack species-level resolution for *Fusarium* (O'Donnell and Cigelnik, 1997). Recently, new *Fusarium*-specific primers targeting the translation elongation factor 1-α (TEF1) gene has been developed and employed to characterise *Fusarium* communities using HTS (Edel-Hermann et al., 2015; Karlsson et al., 2016). This new method enables the study of *Fusarium* in cereals as a community.

Pairing of organic and conventional farms or fields is often used to control for confounding factors such as climate or cultivar (Bernhoft et al., 2010; Granado et al., 2008). However, pairing could select for non-representative farms (Hole et al., 2005). Therefore, we used an agricultural intensity index to directly estimate two of the most important factors differing between conventional and organic systems: pesticide application and amount of nitrogen fertilisation.

In the present study, harvested wheat kernels were collected from pairs of organically and conventionally managed wheat fields in Sweden. Fields were paired to account for variability due to wheat cultivar and geographic location. The *Fusarium* community on the kernels was characterised using amplicon sequencing and real-time PCR. The aims of the study were: 1) to characterise the *Fusarium* community composition and abundance in harvested kernels in Sweden; and 2) to investigate the impact of agricultural factors on the *Fusarium* community in organic and conventional production.

2. Materials and methods

2.1. Sampling and data collection

Twelve pairs of organically and conventionally managed fields in central Sweden were included in the study (Fig. 1). Fields were paired to limit variation due to other factors than cropping system. Two conditions were used to pair the fields: 1) they had to lie close to each other; and 2) they had to contain the same winter wheat cultivar. Paired fields were adjacent or located between 0.5 and 9.5 km from each other and grew either winter wheat varieties 'Olivin' or 'Stava'.

At harvest 2012, farmers were asked to send a sample of harvested kernels from the fields. The farmers were asked to combine several subsamples into a representative sample of the field. Earlier in the season, in July 2012, wheat leaves were collected from the same fields and a number of additional data were collected at the same time (Karlsson et al., 2017). At the start and end of the transect used for leaf sampling, two 0.25 m^2 squares were laid out and weeds and crop plants in each square were cut and the biomass was separated, dried for 24 h at 105 °C and weighed to determine dry mass. The farmers managing the different fields were interviewed about the preceding crops, soil tillage, pesticides, fertilisation and other measures applied in the sampled fields (Table S1).

These data were also used to calculate the agricultural intensification index proposed by Herzog et al. (2006) for each field. The original index includes three indicators: livestock density, amount of nitrogen applied (kg/ha) and number of pesticide applications. Only the two latter were applicable to our study.

2.2. DNA extraction, PCR and 454 sequencing

In total, 18 farmers out of 24 provided a sample of harvested wheat kernels from the fields sampled in July. From each sample of about 1 kg wheat kernels, 100 g were milled with a Grindomix GM 200 (Retsch GmbH, Germany). DNA was extracted from two 100 mg subsamples for each milled sample using the DNeasy Plant Mini kit (Qiagen, Germany) according to the manufacturer's instructions. Kernel material, 300 µl 1 mm glass beads and 530 µl lysis buffer was added to a tube and the samples were run at 30000 min-1 for 40 s on a Retsch MM 400 oscillatory mill. DNA extraction was completed in a QiaCube (Qiagen). Two water controls were included during the DNA extraction. DNA from the two extraction replicates was pooled before PCR.

A portion of the TEF1 gene was amplified using *Fusarium*-specific primers Fa (TCGTCATCGGCCACGTCGACTCT) and Ra (CAATGACGGTGACATAGTAGCG) (Edel-Hermann et al., 2015). PCR was run in 25 µl reactions containing 0.8 ng/µl template, 190 µM of each nucleotide, 2.75 mM $MgCl_2$, primers at 200 nM and 0.12 U/µl polymerase (DreamTaq Green, Thermo Scientific, MA, USA) in PCR buffer. The PCR conditions on a 2720 Thermal Cycler (Life Technologies, Carlsbad, CA, USA) were 3 min at 94 °C, 20–33 cycles of [1 min at