International Journal of Food Microbiology 222 (2016) 30–39

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

The fungal community changes over time in developing wheat heads

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ARTICLE INFO

Article history:
Received 12 November 2015
Received in revised form 14 January 2016
Accepted 24 January 2016
Available online 28 January 2016

Keywords:
Amplicon sequencing
Fusarium
Alternaria infectoria
Mycotoxins
Mycobiome
Next generation sequencing

ABSTRACT

Under normal conditions, wheat is colonized by a multitude of fungi that can have beneficial or adverse effects on plant growth and yield. To study the effect of spraying wheat heads with fungicides on the fungal community from emergence to harvest we applied an amplicon sequencing approach on single wheat heads. The climatic data showed that the spring of 2014 was very dry and without precipitation in the two weeks around flowering. An initial quantitative PCR showed that the total amount of fungal DNA increased during the entire period, without significant difference between sprayed and control wheat heads. Amplicon sequencing of the internal transcribed spacer 2 (ITS2) region showed that operational taxonomic units (OTUs) identified as *Sporobolomyces roseus* dominated in the first weeks, whereas *Alternaria infectoria* OTUs dominated in the last weeks before harvest. The only observed significant difference was that the control wheat heads contained more of the powdery mildew causing *Blumeria graminis* f. sp. *tritici* OTUs compared with the sprayed wheat heads. The dry conditions around flowering most likely also had an effect on *Fusarium* head blight infection as *Fusarium* OTUs were only sporadically encountered.

Analyses of secondary metabolites produced by *Fusarium* and *Alternaria* in the wheat heads confirmed the observations from the amplicon sequencing. Enniatin B was the most frequent contaminant present in four sprayed (49–538 ng/g) and three control (56–355 ng/g) wheat heads. The *A. infectoria* secondary metabolites infectopyrone and 4Z-infectopyrone were however consistently observed in all samples collected the last five weeks before harvest.

1. Introduction

Common wheat (*Triticum aestivum* L.) is one of the most important crops worldwide, only surpassed by maize and rice (FAOSTAT, 2015). Wheat plants are however under continuous attack from pathogenic fungi, bacteria and insects; these have a huge impact on yield and quality. The most important fungal pathogens include the biotrophs *Blumeria graminis* (powdery mildew) and *Puccinia* spp. (rusts), as well as the necrotrophs *Pyrenophora tritici-repentis* (tan spot), *Mycosphaerella graminicola* (Septoria blotch), *Parastagonospora nodorum* (*Stagonospora* blotch) and *Fusarium* spp. (*Fusarium* head blight) (Bockus et al., 2010; King et al., 1983; Parry et al., 1995). Less pathogenic fungi are also very frequently found on wheat, including the black head causing *Alternaria*, *Cladosporium* and *Epicoccum* (Larran et al., 2002). Pink (*Sporobolomyces* spp.) and white (*Cryptococcus* spp.) yeasts are also common saprophytes found on wheat leaves and grains (Fokkema, 1971). Of these fungi *Fusarium* and *Alternaria* are of concern due to the production of bioactive secondary metabolites of which some are classified as mycotoxins (Andersen et al., 2015; Pereira et al., 2014).

The role of the most important wheat pathogens is well studied at single species level, but little is known about the role of the fungal community in disease development. Understanding the complex interactions between host plants and their microbial inhabitants is important for development of sustainable disease management and is therefore a high priority. The mycobiota have traditionally been examined through examining visual symptoms of the plants or by isolating and cultivating fungi using different media and growth conditions (Abildgren et al., 1987; Hocking and Pitt, 1980; Sørensen et al., 2009). Biotrophic fungi and slow growing fungi can however be overlooked in the culture dependent methods. The technical advances in next generation sequencing techniques have enabled culture-independent analyses of the mycobiome (fungi and their genes) (Guttman et al., 2014). This

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http://dx.doi.org/10.1016/j.ijfoodmicro.2016.01.018
0168-1605/

can be studied through a targeted amplicon sequencing approach, where a specific genomic region from fungi is amplified by PCR and sequenced (Ghannoum et al., 2010; Xu et al., 2012).

Internal transcribed spacer regions 1 and 2 (ITS1 and 2) are the preferred targets for amplicon sequencing, because primers have been designed for this region that anneal to nearly all fungi and yield products of appropriate sizes for sequencing (Lindahl et al., 2013). The ITS regions are well represented in reference databases and have also been proposed as the barcode for fungi (Schoch et al., 2012). Comparison of ITS1 and ITS2 amplicon sequencing data from a study of balsam poplar leaf-associated fungal communities showed that ITS1 and ITS2 resulted in a similar pattern in the community composition (Bazzicalupo et al., 2013). The amplicon sequencing approach has been used in studies of the fungal community in samples of wheat leaves (Karlsson et al., 2014) and grains (Nicolaisen et al., 2014). In the study by Karlsson et al. (2014) 235 operational taxonomic units (OTUs) at the species level were identified in wheat leaves, illustrating the rich diversity in fungal colonists in wheat at a given time point.

Wheat plants mature through a series of growth stages until harvest (Zadoks et al., 1974) and over this time interval the fungal community changes. The fungal community in cereals is also affected by application of fungicides, as has been demonstrated in previous studies based on culture-dependent methods (Dickinson, 1973; Dickinson and Wallace, 1976; Magan and Lacey, 1986) and amplicon sequencing (Karlsson et al., 2014; Sapkota et al., 2015; Taheri et al., 2015). Fungicides which have a negative effect on a narrow or wide target group of pathogens can increase the competitive fitness of the remaining resistant pathogens. Most studies of fungal communities in wheat have been performed on composite samples. This reduces the possibility of identifying the communities at a single plant level. In the present study we have therefore examined the development of the fungal community in single wheat heads from emergence until harvest nine weeks later.

2. Materials and methods

2.1. Climate data

Temperature and precipitation data during the growth season were obtained from the weather station 609600 Juvre through the Danish Meteorological Institute (DMI). The weather station is located on the island Rømø, 20 km from the wheat field studied.

2.2. Field management and sampling

The survey was conducted on a conventionally managed winter wheat (variety: Mariboss) field in Southern Denmark (55°10′34″N 8°50′05″E, Fig. 1A). The previous crop was spring oilseed rape and the wheat was sown in early September 2013. The entire field was sprayed with the fungicides Flexity® (0.5 L/ha; active ingredient: Metrafenone; BASF Crop Protection Denmark) on May 5th, 2014 and Viverda® (0.7 L/ha; active ingredients: Boscalid, Epoxiconazol and Pyraclostrobin; BASF Crop Protection Denmark) on May 20th, 2014. The wheat heads emerged in the first week of June (week 24) and 90% of the field was sprayed with Proline® (0.3 L/ha; active ingredient: prothioconazol; Bayer Cropscience Denmark) as illustrated in Fig. 1B. Ten wheat heads were collected from two corner areas (10 × 10 m) in the field (one sprayed with Proline® and one unsprayed control) in weeks 24, 25, 26, 27, 28, 29, 30, 31 and 33 until harvest. The collected wheat heads were stored at −20 °C before analyses. In the following experiments each collected wheat head was considered as one sample. To determine the dry matter content of wheat heads they were weighed and subsequently lyophilized.

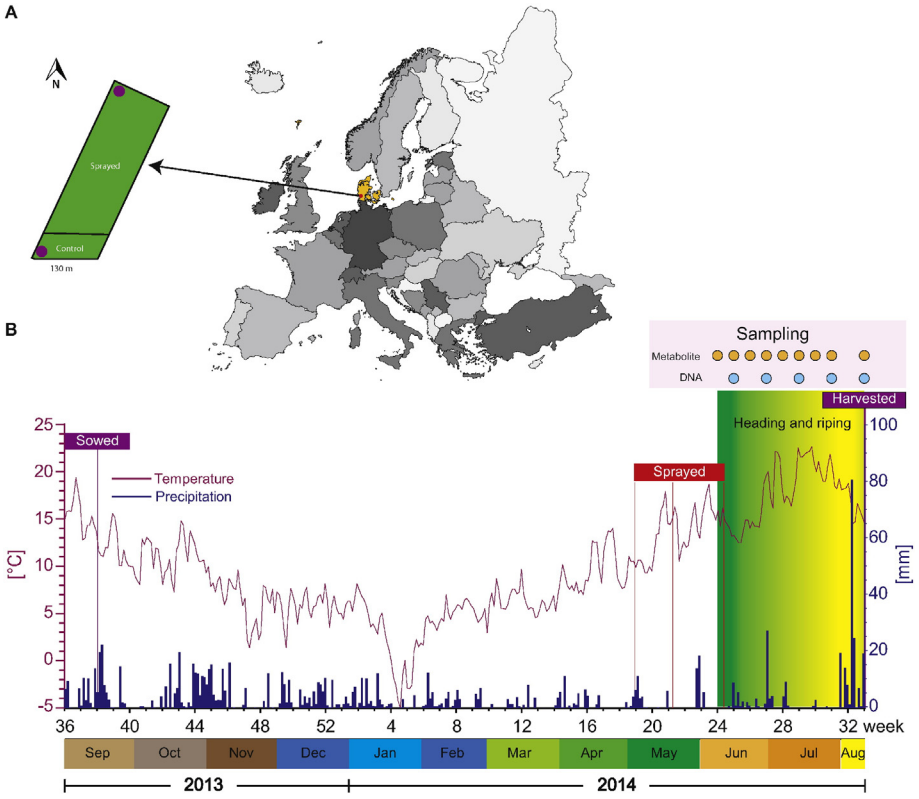

Fig. 1. Geographical localization and climatic conditions. (A) Single wheat heads were collected from a wheat field in Southern Denmark. The majority of the field was sprayed three times with fungicides, while the most southern part of the field was not sprayed the third time when the heads had emerged. (B) The temperature and precipitation data were collected from the nearest official weather station. The wheat was sown in September 2013 and harvested in August 2014. Wheat heads emerged in week 24 and 10 wheat heads were collected from the sprayed and control area every week until harvest in week 32.