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Effect of environmental factors on *Fusarium* population and associated trichothecenes in wheat grain grown in Jiangsu province, China

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ABSTRACT

The present study was performed to identify prevailing *Fusarium* species and the environmental factors affecting their frequencies and the contamination of grain with major mycotoxins in Jiangsu province. The precipitation levels were 184.2 mm, 156.4 mm, and 245.8 mm in the years 2013–2015, respectively, and the temperature fluctuated by an average of 10.6 ± 7.2 °C in 2013, 10.9 ± 7.2 °C in 2014, and 10.6 ± 6.3 °C in 2015. Co-occurrence of deoxynivalenol (DON), 3-acetyldeoxynivalenol (3ADON), and 15-acetyldeoxynivalenol (15ADON) were observed in wheat. The average concentrations of DON were 879.3 ± 1127.8, 627.8 ± 640.5, and 1628.6 ± 2,168.0 μg/kg in 2013–2015, respectively. The average concentrations of 3ADON were 43.5 ± 59.0, 71.2 ± 102.5, and 33.5 ± 111.9 μg/kg in 2013–2015, respectively. We found that the average concentration of DON in wheat was positively correlated with precipitation ($r = 0.998$, $p < 0.01$), and 3ADON was negatively correlated with precipitation ($r = -0.887$, $p < 0.05$). However, there was no correlation between precipitation and 15ADON or nivalenol (NIV). The differences in temperature were not as significant as the differences in rainfall amount over a short time period. Therefore, there were no correlations between temperature and the concentrations of trichothecenes, excluding 3ADON ($r = 0.996$, $p < 0.01$). Our data indicated that *Fusarium asiaticum* is the primary pathogenic fungus prevalent in the Fusarium head blight disease nursery. The trichothecene chemotype composition differed between *Fusarium graminearum* sensu stricto (s. str.) and *F. asiaticum* isolates. The 3ADON chemotype was found only among strains of *F. asiaticum*. The NIV chemotype was not observed among strains of *F. graminearum*, while the 15ADON chemotype represented 100% of the *F. graminearum* strains collected. The results of this study indicated no correlations between environmental conditions and the species or genetic chemotype composition of pathogens in Jiangsu province in 2013–2015.

1. Introduction

Fusarium head blight (FHB) or scab is a significant fungal disease seen in wheat, barley and other small cereal grains globally (Jennings et al., 2004; Marco et al., 2014). Due to the typical humid and warm climate during the flowering stage of wheat (anthesis), there was a serious outbreak of scab in Jiangsu province, a traditional FHB epidemic area located in the lower reaches of Yangtze-Huaihe. Rainfall at anthesis affects FHB incidence, severity, and the production of mycotoxins in wheat grain (Lacey et al., 1999).

Trichothecenes are toxic secondary metabolites produced by the *Fusarium graminearum* species complex (FGSC), which can easily contaminate many cereal grains and the derived animal feed during cultivation or storage. Strains of FGSC typically produce one of the three trichothecene profiles: (i) deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3ADON chemotype), (ii) DON and 15-acetyldeoxynivalenol (15ADON chemotype), or (iii) nivalenol (NIV), its acetylated derivatives, and low levels of DON (NIV chemotype) (Ward et al., 2002). Population subdivision based on the trichothecene genotypes of *F. graminearum* prevalent in Jiangsu province has been inferred from population structure analysis (Qiu et al., 2014). Mattila et al. (2009) reported that 93.5% of isolates in southern Russia possessed the 15ADON chemotype, whereas isolates of *F. graminearum* recovered in Finland and northwestern Russia were exclusively 3ADON producers.

The most well-known and commonly found compound in this mycotoxin family is deoxynivalenol (DON) (Starkey et al., 2007; O'Donnell et al., 2008; Sarver et al., 2011). DON is the most frequently detected trichothecene in grain samples such as wheat, corn, and

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barley, but 3ADON and 15ADON can also be found (Edwards, 2009; Ok et al., 2009). In 2010 and 2012, 100% and 96%, respectively, of wheat harvested from Jiangsu province was contaminated with DON (Ji et al., 2014). Li et al. (2012) reported that 35%, 49%, and 34% of wheat from China was contaminated with 3ADON, 56%, 91%, and 28% in wheat products were contaminated with 15ADON in 2009, 2010, and 2011, respectively.

Therefore, the main goal of this study was to evaluate the naturally occurring *Fusarium* population and associated trichothecenes in grain samples collected in Jiangsu province from 2013 to 2015, as well as to identify environmental factors affecting the species frequency shifts in the populations of these pathogens.

2. Materials and methods

2.1. Acquisition and presentation of meteorological data

Meteorological data were recorded by the automatic weather stations of the National Meteorological Center of CMA (China Meteorological Administration). The data can be found at http://eng.nmc.cn/. To evaluate the average monthly time courses of temperature and precipitation across Jiangsu province, 26 stations that delivered data during the entire period of the study, covering all regions of the country were selected. Means of average monthly temperatures and cumulative monthly precipitation were calculated for 5 months (January, February, March, April, and May) between 2013 and 2015.

Data on wheat anthesis between 2013 and 2015 were determined for the different regions of Jiangsu province in a previous study (Geng et al., 2012). Cumulative precipitation around the time of wheat anthesis was calculated starting 7 days before anthesis and ending 7 days post-anthesis for the 26 stations, taking the different dates of anthesis in the regions into account.

2.2. Fungal material

2.2.1. Sampling of infected wheat and isolation of fungal strains

For sampling of infected wheat, a total of 450 wheat samples were collected from 26 stations in the autumn from 2013 to 2015. The samples were taken from three areas in the middle to the low valley of Huaihe River and Yangtze River of China, i.e., northern, central, and southern regions of Jiangsu province (Fig. 1). For each sample, at least three aliquots of 200 g were obtained (EC NO. 401/2006). Each 200 g of a sample was ground to a fine powder and stored in paper bags at room temperature until further analysis.

For isolation of fungal strains, individual seeds from wheat heads were dislodged and surface sterilized before plating on potato dextrose agar, as described previously (Zhang et al., 2012).

2.2.2. Determination of species and genetic chemotypes

In 2013, 2014, and 2015, DNA extraction was performed according to Leslie and Summerell (2006). DNA from all isolates was amplified by PCR using Fg16F/R primers, which produce polymorphic products (400–500 bp) from DNA from members of the FGSC (Nicholson et al., 1998). Single and multiplex PCR assays were performed for the detection of trichothecene genotypes. Chemotypes of the FGSC isolates were determined using the specific primers described by Li et al. (2005). Another two primer sets, Tri303F/Tri303R and Tri315F/Tri315R, which target the Tri3 gene (Jennings et al., 2004), were used to further characterize DON chemotypes of the *F. graminearum* sensu stricto (s. str.) complex as 3ADON or 15ADON. Multiplex PCR assays developed by Wang et al. (2012) were performed using primer pairs based on sequences of the Tri11 gene, a key enzyme in the pathway leading to T-2, DON, NIV, 3ADON, and 15ADON biosynthesis in *Fusarium* species (Alexander et al., 1998). These primers generated a 279 bp fragment for the 15ADON chemotype, a 334 bp fragment for the 3ADON chemotype, and a 497 bp for the NIV chemotype.

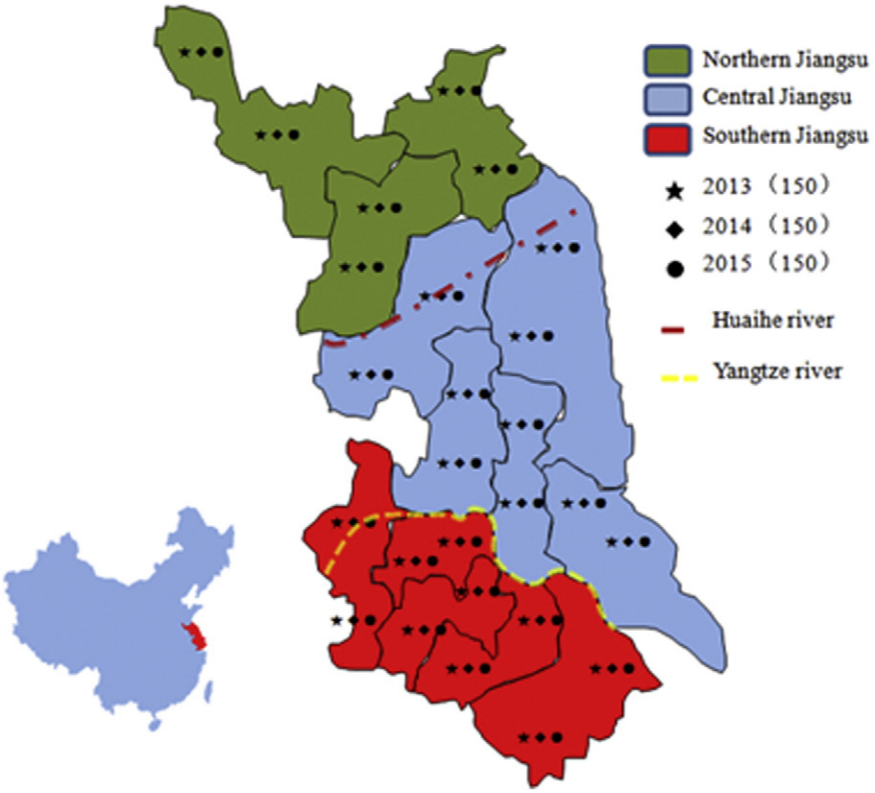

Fig. 1. Map of Jiangsu showing the locations of different studied regions for sample collection.