

Biogeography of *Fusarium graminearum* species complex and chemotypes: a review

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Differences in the geographic distribution of distinct trichothecene mycotoxins in wheat and barley were first recorded two decades ago. The different toxicological properties of deoxynivalenol (DON), nivalenol (NIV) and their acetylated derivatives require careful monitoring of the dynamics of these mycotoxins and their producers. The phylogenetic species concept has become a valuable tool to study the global occurrence of mycotoxin-producing *Fusarium* species. This has revolutionised our views on the terrestrial distribution of trichothecene-producing *Fusaria* in the context of agronomics, climatic conditions, and human interference by the global trade and exchange of agricultural commodities. This paper presents an overview of the dynamics of the different trichothecene-producing *Fusarium* species as well as their chemotypes and genotypes across different continents. Clearly not one global population exists, but separate ones can be distinguished, sometimes even sympatric in combination with different hosts. A population with more pathogenic strains and chemotypes can replace another. Several displacement events appear to find their origin in the inadvertent introduction of new genotypes into new regions: 3-acetyl-DON-producing *F. graminearum* in Canada; 3-acetyl-DON-producing *F. asiaticum* in Eastern China; 15-acetyl-DON *F. graminearum* in Uruguay; and NIV-producing *F. asiaticum* in the southern United States.

Keywords: *Fusarium*; mycotoxins; trichothecenes; population dynamics

Introduction

The occurrence of mycotoxins in agricultural products is subject to extensive research across the globe as these secondary metabolites occur in many staple crops. Because mycotoxins are detrimental for consumers of contaminated food and feed, their occurrence in commodities is regulated in many countries. Among the most important fungal species implicated in the production of these fungal compounds are members of the genera *Aspergillus*, *Fusarium* and *Penicillium*. Major toxin producers in the genus *Fusarium* belong to the *Fusarium fujikuroi* and *F. graminearum* species complexes, FFSC and FGSC, respectively.

Members of the FFSC are capable of producing fumonisins, a range of chemically related mycotoxins that are implicated in several serious diseases in animals and humans, including encephalomalacia in horses, pulmonary oedema in pigs as well as oesophageal cancer in humans. Major fumonisin producers are *F. verticillioides*, a major contaminant of maize, and *F. proliferatum*, a polyphagous species occurring on many different crops. Several other FFSC members harbour the entire fumonisin gene cluster involved in the biosynthesis of this secondary metabolite and some were shown to produce varying amounts of fumonisins (Proctor et al. 2013).

This review focuses on the FGSC, where at least 16 species have been recognised using multi-locus sequence typing (O'Donnell et al. 2000, 2004, 2008; Starkey et al. 2007; Yli-Mattila et al. 2009; Sarver et al. 2011). These species cause *Fusarium* head blight (FHB) in small grain cereals that can lead to yield losses exceeding 50% (Parry et al. 1995). In addition, they are capable of producing a class of mycotoxins known as trichothecenes. Trichothecenes are sesquiterpenoid molecules of which many variants are known. The most important type B trichothecenes are deoxynivalenol (DON), nivalenol (NIV) and derivatives of DON and NIV, particularly 3-acetyl and 15-acetyl deoxynivalenol (3ADON and 15ADON) as well as 4-acetyl nivalenol (4ANIV). The genes encoding for the enzymatic pathway are largely clustered and have been studied in detail. The type of trichothecene produced by an isolate can be predicted based on genetic markers derived from this gene cluster.

The different mycotoxins have different toxicological properties. NIV is more toxic than DON to humans and domestic animals and a stricter limit for the temporary tolerable daily intake of NIV (0.7 µg kg⁻¹ body weight; for DON, 1 µg kg⁻¹) has been issued by the European Scientific Committee for Food (Schothorst & van Egmond 2004; EFSA CONTAM Panel 2013).

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In a study by Yoshizawa and Jin (1995) on the (co-) occurrence of different trichothecenes in wheat and barley in Japan, regional differences were observed. 15ADON was the major mycotoxin in the North part of the country, NIV in the central regions, whereas 3ADON alone or in combination with NIV was observed in southern Japan. Phylogenetic species recognition in FGSC together with this first report on the geographic structuring of trichothecenes has initiated multiple surveys on the occurrence of trichothecenes and population studies on trichothecene-producing *Fusarium* species.

The differences in toxicity of each of the individual trichothecenes warrants surveillance of the (co-) occurrence of these mycotoxins and their producers. Further investigation and active surveillance of both pathogens and their toxins in cereal food and feed chains is required worldwide. The purpose of this paper is to present an overview on these surveys on members of the FGSC across different continents focusing on the different chemotypes, 3ADON, 15ADON and NIV.

Populations of *Fusarium* isolates studied were (preferentially) obtained by hierarchical sampling in wheat, barley and maize growing areas around the globe. These populations are then screened with species-specific primers preferably based on multi-locus genotyping to investigate differences in the species composition of these geographic diverse populations. Subsequent screening with PCR primers that distinguish between chemotypes, e.g. the genetic basis for the production of different trichothecenes, allows discrimination between 3ADON, 15ADON or NIV production. In addition, neutral genetic markers, such as VNTRs (variable number of tandem repeats), AFLPs (amplified fragment length polymorphism) or single nucleotide polymorphism detected by whole genome sequencing provide insight in the genetic constitution of individuals within and between populations.

Distribution of FGSC species and chemotypes in Asia

Distribution in Japan

The distribution of *Fusarium* species on wheat and barley across Japan showed a clear geographic distinction. In the north, primarily *F. graminearum* was observed, while the predominant species in the south was *F. asiaticum* (Suga et al. 2008). There was a strong correlation with the mean temperature, with *F. graminearum* observed in prefectures with a mean temperature <15°C and *F. asiaticum* in those with a mean temperature > 15°C (Suga et al. 2008). In the central part of Japan both populations were apparently co-occurring, but no gene flow between the two species was observed (Suga et al. 2008). The above-mentioned earlier study by Yoshizawa and Jin (1995) linked the north with 15ADON production, while NIV and 3ADON were the mycotoxins observed in the warmer south. Using specific

primers for 3ADON, 15ADON or NIV producers (Ward et al. 2002), Suga et al. (2008) showed that *F. graminearum* strains were either 3ADON or 15ADON type, while the majority of *F. asiaticum* strains was of the NIV type. No correlation between host origin and fungal species was found (Suga et al. 2008).

Distribution in China

In a large survey in Southern China on barley the primary source of FHB was *F. asiaticum* (> 90%; 1706/1894) and only limited numbers of other members of FGSC were encountered: *F. graminearum* (2.7%; $n = 45$); *F. meridionale* (1.4%; $n = 27$) or other FGSC species (3.5%; $n = 68$). The majority of these non-*F. asiaticum* isolates were found in the upper valleys of the Yangtze River (Yang et al. 2008). *F. asiaticum* was also the major component of the FHB complex on wheat grown in Southern China (267/275, approximately 97%). In contrast in the North/Northeast of China, with 129 out of 169 isolates (about 76%) *F. graminearum* was found to be the predominant species (Zhang et al. 2012). This is in good agreement with results obtained by Gale et al. (2002) and Waalwijk et al. (2008) who found *F. asiaticum* exclusively in fields in the southern provinces Zhejiang and Hubei, respectively.

In barley, isolates from the Southwest, e.g. the upper valleys of the Yangtze River, were mostly NIV producers, while in the lower regions predominantly 3ADON producers were encountered. In both regions the frequency of 15ADON producers was negligible. A more complex situation was observed in wheat, where *F. graminearum* isolates were exclusively of the 15ADON type, while *F. asiaticum* could be separated in NIV producers ($n = 97$) or 3ADON producers ($n = 171$). Again 15ADON-producing *F. asiaticum* strains were very infrequently observed (Zhang et al. 2012).

A strong correlation is observed between the incidence of *F. asiaticum* or *F. graminearum* with the predominant rotation scheme. In regions, where wheat/maize is the most common rotation, e.g. the North of China, *F. graminearum* is widespread. In contrast, in areas where wheat is mainly grown in rotation with rice, mainly *F. asiaticum* was found (Zhang et al. 2012). This is in agreement with the notion that *F. asiaticum* has a preference for rice (Lee et al. 2009, 2010). When all three crops are grown in large acreages, such as in Sichuan province, both species are observed. In another study (Zhang et al. 2007), climatic factors were included: *F. graminearum* is mainly found in cooler areas (< 15°C) and *F. asiaticum* in warmer regions (> 15°C). Not unexpectedly, these regions coincide with wheat/maize and wheat/rice rotations. A similar geographic distribution of the two species was observed in Japan, although maize cultivation has completely disappeared there in the last three decades (Index Mundi 2014).

To investigate whether the above observations are (in part) caused by the role of the different trichothecenes in the pathogenicity of *Fusarium* against maize, wheat and barley, the substructure of barley and wheat populations was determined with assumed neutral markers described by Suga et al. (2004). These VNTRs, when tested on a subset of barley isolates, showed varying levels of genetic diversity with allele numbers ranging from one to 14 (Zhang, Zhang, van der Lee, Chen, Arens, et al. 2010). Further analyses with the most informative VNTRs revealed a clear disequilibrium in allele frequencies between different regions. Whereas some alleles were found in the upper, middle as well as the lower regions of the Yangtze River, others were (exclusively) confined to one or two of these areas. In Sichuan province and in Chongqing the largest variation and the highest number of unique alleles was found (Zhang, Zhang, van der Lee, Chen, Arens, et al. 2010) was found. Taking together the range of species, chemotype assortment and VNTR allele diversity, *Fusarium* populations collected from the Southwest of China harbour the largest diversity.

The observed genetic structure of *F. asiaticum* populations from barley is indicative of limitations in genetic exchange. These might be of biological, physical and/or agronomic nature. We found that different genotypes of the same species as well as different FGSC species co-occur in different sampling sites (Zhang, Zhang, van der Lee, Chen, Arens, et al. 2010; Zhang, Zhang, van der Lee, Chen, et al. 2010). This suggests that crosses between genotypes might occur. However, the clear genetic delineation of a population of *F. graminearum*, producing 15ADON, and two populations of *F. asiaticum*, producing respectively 3ADON or NIV, strongly suggest that a biological barrier has limited genetic exchange between these populations. Furthermore, as members of the FGSC are homothallic they can complete a sexual cycle without a partner, which allows local fixation of alleles and/or chemotype (Zhang et al. 2012).

Physical or geographic barriers can be observed in Sichuan and Chongqing, the regions with highest genetic diversity. Sampling sites in these provinces are located in the mountain rich parts of China, with elevations exceeding 1000 m. Valleys that are separated by such ridges may form ecological niches, where genetic exchange occurs infrequently. In addition, the influx of invading genotypes will be restricted. The genetic structuring in Hubei province versus the lower Yangtze River region (Zhejiang, Jiangsu and Shanghai) showed that two alleles observed in Hubei were not found in the lower valleys and five alleles from the lower valleys were not found in Hubei. This may be caused by the small-scale farming practise frequently found in these regions (Zhang, Zhang, van der Lee, Chen, Arens, et al. 2010).

The wider species diversity in combination with the higher genetic diversity in the Southwest of China suggest

this resembles the original, diverse FGSC population in China (Yang et al. 2008). The gradient of NIV versus DON chemotypes along the Yangtze River was suggested to have been caused by a shift in the population. However, the number of VNTR alleles in the middle and lower valleys advocates that this invasion is slow and may be potentially masked by outcrossing between old and new populations (Zhang et al. 2012).

For a better understanding of the genetic subdivision and the dynamics of *Fusarium* in cereal-growing areas, populations were also characterised for different phenotypic traits. These included: (1) growing rate *in vitro*, (2) the incidence of infected spikelets, (3) biomass production, (4) mycotoxin production, (5) conidiation, (6) conidium size and (7) fungicide resistance. Significant differences were observed between all populations. Strikingly, 3ADON-producing *F. asiaticum* were superior to NIV-producing *F. asiaticum* in all seven traits, and this aggressive 3ADON-producing *F. asiaticum* population is spreading from East to West (Zhang et al. 2012).

Distribution in South Korea

In South Korea, the vast majority of isolates from rice belonged to *F. asiaticum* and the predominantly observed chemotype (95%) in these isolates was the NIV chemotype (Lee et al. 2010). In contrast, the dominating species on maize was *F. graminearum*. The predominant chemotype among the maize isolates varied according to region. The DON chemotype was found more frequently (66%) than the NIV chemotype in the North-Eastern Gangwon province, whereas the NIV chemotype (70%) was predominant in central Chungbuk province. Recently, among 568 isolates of FGSC from maize four species were identified: *F. graminearum* (75%), *F. boothii* (12%), *F. asiaticum* (12%) and *F. meridionale* (1%), with the first two species producing DON while the others were NIV producers (Lee et al. 2012). AFLP results suggest that the DON-producing *F. graminearum* may have been introduced – along with maize seeds – from the United States, while the *F. boothii* and *F. asiaticum* seem to originate from local population occurring on rice (Lee et al. 2012).

Distribution in Iran

Another clear case of geographic substructuring was observed in Iran, where in Ardabil province in the western part of the country 125 of 129 isolates were identified as *F. graminearum*, among which 121 had the 15ADON chemotype. In contrast, in Golestan, Eastern Iran, the population was much more diverse with nine species among 26 isolates. Moreover, out of 12 *F. graminearum* isolates, 11 had the NIV chemotype (Davari et al. 2013). This is in good agreement with Haratian et al. (2008) who

found a majority of NIV producers among 57 isolates from the North-east of Iran. In a third study, geographic limitations between both regions in Iran were suggested to explain similar observations (Malihpour et al. 2012). Crop rotation with rice may also provide part of the explanation as NIV producers of *F. asiaticum* as highly dominant in rice growing areas in Korea (Lee et al. 2009). A shift comparable with the situation in China may occur in Iran as well.

Distribution of FGSC species and chemotypes in Europe

Surveys on FHB and mycotoxins have been performed in many countries in Europe over the last decades. For example, *F. culmorum* was the main cause of FHB in the Netherlands before the year 2000, but appears to be replaced by *F. graminearum* (Waalwijk et al. 2003). Similarly, *F. graminearum* was sparsely observed in wheat and barley samples in Denmark, dating back as far as 1957 (Nielsen et al. 2011). This was in sharp contrast with recent samples where *F. graminearum* is the major FHB constituent. (Nielsen et al. 2011, 2012). Beyer et al. (2014) suggest that shifts from NIV-producing *F. culmorum* strains to 15ADON-producing *F. graminearum* in Luxembourg are related to the better competitiveness of the latter species under humid conditions.

The dominance of *F. graminearum* was also observed in other European countries, e.g. France (Waalwijk et al. 2008), Germany (Talas et al. 2011) and Italy (Prodi et al. 2009, 2011). Boutigny et al. (2014) identified *F. graminearum* as the dominant FGSC species on wheat, barley as well as maize in France. These populations were predominantly 15ADON, but some NIV types were observed. This chemotype occurred only on wheat and maize (but not on barley) and this was only seen in samples that originated from the south of France. In Italy, the 15ADON genotype was predominantly found in regions with a cool winter climate (Prodi et al. 2009, 2011). These findings were confirmed in another study that showed mostly 15ADON-producing *F. graminearum* in Italy (Somma et al. 2014). In wheat fields in England and Wales mainly 15ADON producers (72/76) occurred among *F. graminearum* isolates (Jennings et al. 2004).

In Finland as well as in the northern part of Russia exclusively the 3ADON chemotype of *F. graminearum* was observed (Yli-Mattila et al. 2009). In contrast, in the southern part of Russia only 15ADON isolates were encountered. This is in good agreement with surveys in the same region, where in Ardabil, in the north-west of Iran, 15ADON producers were obtained (Davari et al. 2013).

Unfortunately, the absence of analyses on European populations using neutral markers such as VNTRs has

hampered thorough interpretations on dynamics of FHB pathogens on this continent.

Distribution of FGSC species and chemotypes in North America

In Canada a migration event was reported, where 3ADON-producing *F. graminearum* was reported to replace 15ADON-producing *F. graminearum* (Ward et al. 2008). The authors of this study analysed the chemotype of 492 *F. graminearum* isolates from three sampling periods (1998–2004) and found that approximately 25% of these were of the 3ADON chemotype and 75% were of the 15ADON chemotype. They observed a pronounced longitudinal cline with 100% 3ADON in the east and < 10% 3ADON in the west. In addition, in the Western provinces the contribution of 3ADON increased 14-fold between 1998 and 2004. Subsequent VNTR analyses revealed that these chemotypes represent genetically different populations that coincide with different phenotypic attributes: 3ADON populations produce more and larger conidia, grow faster and accumulate more trichothecene than do 15ADON producers. When these features are also expressed under field conditions, this can account for enhanced fitness. In turn this can lead to rapid displacement of the 15ADON chemotype, in turn leading to a more toxic population in North America (Ward et al. 2008). Similar results were obtained in the upper Midwest of the United States, where besides a predominate presence of 15ADON producers (approximately 95%) some isolates with 3ADON chemotype also were observed (Gale et al. 2007). Using RFLP fingerprinting these isolates could be clustered into three different populations, where the 3ADON population could not be distinguished from a collection of Italian strains. Together with the local occurrence of this population in the United States, the authors speculate that it may have been introduced from Europe (Gale et al. 2007). A comparison between an ‘old’ (1980–2000) and a ‘new’ (2008) population showed a 15-fold increase in the 3ADON (3% > 44%) while the 3ADON producers caused more disease and produced higher amounts of DON than did 15ADON producers (Puri & Zhong 2010).

The so-called ‘Northland population’ represents another population that has the 3ADON genotype, but does not seem to produce any of the known trichothecenes on inoculated spikelets (Gale et al. 2010). Indeed, chemical analyses demonstrated that members of this population produce a novel hitherto unknown trichothecene (Adam, personal communication). A more complex situation is observed in the southern United States where an additional population of NIV producers was identified as well as the so-called Gulf Coast population that consists of 3ADON producers (Gale et al. 2011). Moreover these authors describe the first appearance of (NIV-producing) *F. asiaticum* in the United States.

The occurrence of *F. asiaticum* seems to overlap the rice-growing areas in the southern United States (Gale et al. 2011). Another study in the eastern United States showed a North/South gradient of 3ADON producers in an otherwise overwhelming 15ADON population (Schmale et al. 2011). The appearance of 3ADON producers was also observed in barley, where an 11-fold increase was noted in 2008 compared with the period 1997–2000 (Burlakoti et al. 2011). In view of these clear geographic substructures, the hypothesis that the FHB complex in North America consisted of a single pan-continental population must be rejected.

Distribution of FGSC species and chemotypes in South America

Studies on FHB severity in Latin America have been mainly performed in the Southern Cone, the moderate regions of Northern Argentina, South Brazil and Uruguay, the major grain-growing areas of the continent. In Argentina, the main causal pathogen appears to be *F. graminearum* that has the 15ADON genotype (e.g. Reynoso et al. 2011; Castanares et al. 2014; Malbran et al. 2014). The same results were obtained in Southern Brazil (Scoz et al. 2009; Astolfi et al. 2011). Interestingly, variable numbers of NIV producers were also identified (Scoz et al. 2009; Sampietro et al. 2010, 2011; Umpierrez et al. 2013). Using MLST (multi-locus sequence typing), most of these isolates were identified as *F. meridionale* (Scoz et al. 2009; Astolfi et al. 2011; Sampietro et al. 2011). A small but significant increase of 3ADON producers from 29% to 49% was observed in Argentina between 2001 and 2004 (Alvarez et al. 2009). These authors observed high genetic diversity, but no regional effects in Argentina. In Uruguay, on the other hand, significant differences between western and eastern provinces were observed (Umpierrez et al. 2013). The NIV type predominated in the new wheat-producing areas in the east, where also the production of rice is common. Interestingly, the majority of these NIV producers turned out to be *F. asiaticum* isolates.

FGSC members were also recovered from maize (Sampietro et al. 2010, 2011/2012). However, these isolates belong to *F. meridionale* and *F. boothii*, species that are supposed to be endemic in Latin America (Aoki et al. 2012). All these Argentinian *F. meridionale* isolates have the NIV genotype, while all *F. boothii* strains have the 15ADON genotype (Sampietro et al. 2010). In summary, 15ADON-type *F. graminearum* strains seem associated with wheat, on maize *F. meridionale* with the NIV type is common, while on rice NIV-type *F. asiaticum* occurs (Del Ponte et al. 2013).

Distribution of FGSC species and chemotypes in Africa

The number of surveys on FHB and FGSC diversity in Africa is limited. Wagacha et al. (2010) reported on the

occurrence of 19 different species on wheat in Kenya, with *F. chlamyosporum*, *F. boothii*, *F. poae*, *F. scirpi*, *F. arthrosporioides* and *F. graminearum* accounting for 80% of the infections. In another study, 31 isolates from wheat seeds from Ethiopia were identified as a new species, named *F. aethiopicum*, which produces 15ADON (O'Donnell et al. 2008). MLST suggests that this species together with sister species *F. acaciae-mearnsii* and NRRL 34461 *Fusarium* sp. may be endemic to the Southern Hemisphere (e.g. Africa or Australia). All the *F. aethiopicum* strains were isolated in Ethiopia, while *F. acaciae-mearnsii* and NRRL 34461 isolates were isolated from South Africa (O'Donnell et al. 2000).

A more extensive study was performed in South Africa (Boutigny et al. 2011), where 560 FGSC isolates from wheat, barley and maize were typed. More than 85% of the isolates from wheat or barley were *F. graminearum* with the 15ADON type. In contrast, on maize only *F. boothii* (15ADON) was found, suggesting that the local *F. graminearum* population has limited ability to attack maize.

Distribution of FGSC species and chemotypes in Australia and New Zealand

Little information is available about the situation in Australia and New Zealand. In a recent outbreak of FHB of wheat in Western Australia, *F. graminearum* was involved next to other *Fusarium* species, while both DON and 3ADON were detected (Tan et al. 2012). Also the co-occurring *F. pseudograminearum* proved capable of DON production (Obanor et al. 2013).

Both 15ADON and NIV-producing *F. graminearum* strains and NIV-producing *F. cortaderiae* strains were reported from New Zealand (Lauren et al. 1992; Monds et al. 2005). Reports on maize in New Zealand also hint at the presence of *F. graminearum* next to other species, while in the crop both NIV and DON have been observed (di Menna et al. 1997; Hussein et al. 2002).

Conclusions

The FGSC is a very dynamic complex of morphologically related species that occur in all cereal-growing regions around the globe. Different members show varying levels of host preference, namely *F. asiaticum* on rice, *F. boothii* and *F. meridionale* on maize, whereas *F. graminearum* is encountered on wheat, barley as well as maize. Crop rotation involving these hosts may influence population dynamics, especially since it was shown that NIV and DON act as virulence factors on wheat, while only NIV has some impact on maize (Maier et al. 2006).

We have listed many examples where multiple species of the FGSC coexist at the same location. Yet they appear

genetically and biologically different and must be regarded as separate populations that live sympatric in fields without genetic exchange. Therefore it is important to identify the members on the species level, preferentially by MLST, before population genetic analysis can be performed. *F. graminearum* is found all over the world and appears to be an increasingly dominant component of the FHB complex, particularly in North-West Europe.

F. graminearum isolates around the world show strong a geographic substructure. Even populations that are geographically close can show distinct substructuring. Therefore *F. graminearum* should be considered as a meta-population consisting of many relatively independently developing populations that show demographic stochasticity. The notion that *F. graminearum* isolates around the world belong to one and the same population must therefore be rejected.

Species of the FGSC of the NIV chemotype were found in nearly all wheat-growing areas and since this mycotoxin is more detrimental for consumers, this finding prompts future monitoring for the presence of this toxin in food and feed commodities.

In this paper we listed a number of displacement events that appear to find their origin in the inadvertent introduction of new genotypes into new regions: 3ADON *F. graminearum* in Canada; 3ADON *F. asiaticum* in Eastern China; 15ADON *F. graminearum* in Uruguay; and NIV-producing *F. asiaticum* in the southern United States. With the anticipated changes in climate, crop production will shift to other climatic zones, so further displacements and migration events are likely to occur in the (near) future. This may have a profound impact on toxicity and aggressiveness and consequently increased difficulties to control FHB. To monitor these changes effectively we suggest building a global database in which isolates with GPS data and host are analysed based on the multi-locus genotyping (Ward et al. 2008) in combination with the VNTR screening and potentially whole genome sequencing. This would facilitate early detection of trends and potential new risks originating from changes in the trichothecene producing *Fusarium* species.

The first report on displacement of *F. culmorum* by *F. graminearum* was done in 2003 (Waalwijk et al. 2003). At that time, we suggested that increased maize acreage, the ability to produce ascospores and/or climate changes favouring *F. graminearum* that has a higher temperature optimum could explain the shift from *F. culmorum* to *F. graminearum* as the major FHB pathogen in the Netherlands. The introduction of *F. graminearum* into Western Europe seems a very recent event. In a historical archive, covering more than 160 years of wheat samples, this species was first observed in the late 1980s, while *F. culmorum* was encountered repeatedly since the 1840s (BA Fraaije, pers. communication).

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