

Article

DNA-Based Quantification of *Fusarium* Species in Winter Wheat Grain in Poland from 2014 to 2017 and 2019

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Abstract

This study investigates the connection between meteorological variables and the presence of *Fusarium* species across 26 grain sampling sites over five years. We analyzed average temperatures for May, June, and July, which showed substantial year-to-year fluctuations, with the most significant deviations in 2019 and the smallest in 2014. Precipitation also varied greatly each year, with notable differences between 2014 and 2019. The measurement of *Fusarium* DNA revealed its presence in most samples, with peak levels observed in southeastern and northwest Poland. Five *Fusarium* species were identified, with *F. graminearum* s.s. and *F. poae* being the most prevalent, depending on the year. The geographic distribution of *Fusarium* DNA demonstrated significant regional differences, with the highest levels found in specific provinces each year. Statistical analysis indicated significant correlations between DNA levels of *F. avenaceum*, *F. culmorum*, and *F. graminearum* s.s. Principal Component Analysis (PCA) uncovered strong relationships among these species, especially in samples from 2016 and 2017. Additionally, we observed a positive correlation between *Fusarium* DNA levels and precipitation during flowering and ripening stages, and a negative correlation with temperature during stem elongation and milk-dough phases. These findings highlight the complex interactions between climate factors and *Fusarium* presence in winter wheat grain, providing insight into environmental influences on fungal ecology in agricultural systems.

Keywords: DNA; *Fusarium*; *F. graminearum*; Fusarium head blight; wheat; weather conditions



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1. Introduction

Fusarium head blight (FHB), also known as scab, is a severe wheat disease (*Triticum aestivum*) in Europe and other regions caused by fungi of the genus *Fusarium* [1]. Several species of *Fusarium* can cause FHB, including *Fusarium graminearum* and *Fusarium culmorum*. These fungi infect the developing wheat spikes, causing the grain to become discolored and shriveled, thereby reducing the yield and quality of the crop. The presence of mycotoxins produced by *Fusarium* species in infected grains can also pose a risk to human and animal health. The two species mentioned above are identified as producers of deoxynivalenol (DON), nivalenol (NIV), and zearalenone (ZEN). Other *Fusarium* species that cause FHB include *F. avenaceum*, a producer of moniliformin, enniatins, and beauvericin (BEA); *F. poae*, a producer of diacetoxyscirpenol (DAS), NIV, and BEA; *F. langsethiae* and *F. sporotrichioides*, producers of T-2 toxin, HT-2 toxin, and DAS, also occur [2–7]. Among these species, *Fusarium graminearum* and *F. culmorum* are highly pathogenic to wheat, causing severe

epidemics of FHB. The other species are moderately or weakly pathogenic; however, they may also contaminate wheat grain with mycotoxins [8–11].

Currently, *F. graminearum* is regarded as the primary species responsible for FHB in Europe and other regions, due to its high virulence and ability to produce significant levels of mycotoxins. Phylogenetic studies of the *F. graminearum* strain collection showed that *F. graminearum* is a species complex consisting of 11 distinct species [12–14]. The most common species worldwide within the *F. graminearum* species complex (FGSC) is *F. graminearum* sensu stricto. In Europe, *F. graminearum* s.s. was isolated almost exclusively [15–17]. Only single isolates of other species (*F. boothii*, *F. cortaderiae*) were found, as shown in the analysis of the influence of climate on the distribution of FGSC species by Backhouse [18] and in the bibliographic analysis by Del Ponte et al. [19].

The composition of *Fusarium* species infecting wheat varies based on the climate of the wheat-growing region. In Europe, different species are more common in the North-east, where *F. avenaceum*, *F. culmorum*, and *F. graminearum* s.s. each make up roughly the same portion, compared to the Southwest of the continent, where *F. graminearum* s.s. is predominantly found [2,3,20–23].

The composition of the species changes over time, influenced by climate warming and shifts in the acreage of major cereal crops, such as an increase in the area of maize [24–27]. The primary reported effect of these factors is an increase in the frequency of *F. graminearum* and a decrease in *F. culmorum* [28–30]. Climate warming could favor *F. graminearum* s.s. because it has a higher optimal temperature for growth than *F. culmorum*. Additionally, two separate populations of *F. graminearum* s.s. (Eastern European and Western European) were identified [31]. The authors found differences that could result from different evolutionary paths, including environmental adaptation. Unlike *F. culmorum*, *F. graminearum* s.s. is a significant pathogen on maize and can survive on maize debris [26,32]. It is worth noting that in Poland, the area used for maize cultivation has expanded significantly, from 38,300 hectares (including 59,000 hectares of grain maize) in 1990 to 1,842,960 hectares (including 1,222,480 hectares of grain maize) in 2025 [33–35].

Frequencies of *Fusarium* species infecting wheat heads in Poland from 1985 to 2009 were summarized by Stępień and Chelkowski [36]. In 1985, *F. avenaceum* and *Microdochium nivale* were the dominant species, with *F. culmorum* as the third most common. By 2009, *F. graminearum* became dominant, and *F. culmorum* ranked second, with its frequency about half that of *F. graminearum*. The rise in *F. graminearum* was apparent; however, the differences between certain years were statistically significant. According to various studies conducted in Poland after 2010, *F. graminearum* is now the most prevalent species of *Fusarium* [37–39]. A higher frequency of *F. culmorum* was noted only in certain studies [40,41]. Other species commonly detected include *F. avenaceum* and *F. poae* [39,42].

There has been a recent rise in FHB cases in Poland, raising concerns among farmers and researchers about its effects on food safety and security [43–45]. This disease presents a significant risk in areas with high humidity and rainfall, as these conditions foster the growth and spread of *Fusarium* fungi [39,42,46,47]. In Poland, farmers and researchers use various strategies to reduce the impact of FHB. These include planting resistant cultivars, adopting integrated disease management practices, and developing new technologies to detect and lower mycotoxin contamination [48,49]. However, FHB still presents a significant challenge for the Polish cereal crop industry, which must meet the stricter limits on *Fusarium* toxin levels in cereal grain coming into effect in 2024 [50]. Ongoing research and development efforts are essential to address this problem.

The study aimed to assess the diversity of *Fusarium* species infecting wheat kernels across different regions of Poland from 2014 to 2019. Data from 2018 are unavailable due to a temporary suspension of project funding.

2. Materials and Methods

2.1. Wheat Grain Samples

Wheat grain samples were collected during the harvest seasons of 2014–2017 and 2019. They came from 26 experimental stations of COBORU (the Research Centre for Cultivar Testing; <https://coboru.gov.pl/>; accessed on 18 September 2025), located in various regions of Poland (Figure 1). Two winter wheat cultivars, moderately susceptible to FHB—‘Arkadia’ and ‘Bamberka’—were included. ‘Bamberka’ was replaced in 2017 by ‘Hondia,’ a variety that is moderately resistant to FHB [51]. According to COBORU data (<https://coboru.gov.pl/>), these three cultivars were among the most widely grown in Poland during the study years. Winter wheat was cultivated on 10 m² plots in two replications with moderate nitrogen input (an average of 90 kg/ha of N), without chemical disease control except for seed treatment with fungicides. Herbicides and insecticides were applied as needed. The grain was harvested using a combine harvester. Samples were taken from the mixed grain collected from two plots.

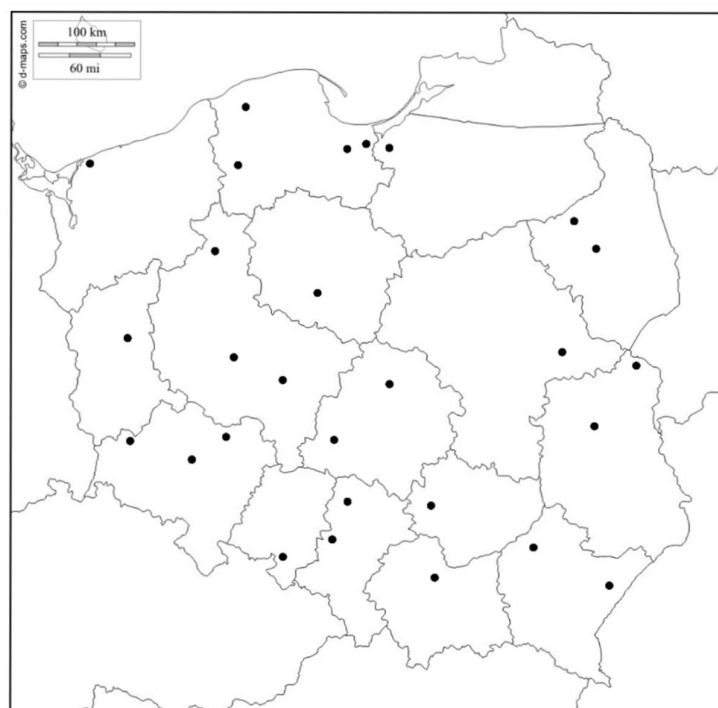


Figure 1. Map of Poland indicating the locations of winter wheat plots where grain samples were collected from 2014 to 2017 and in 2019.

The number of locations where samples were collected varied each year depending on grain availability (Table 1). Despite this, most regions were sampled every year. Exceptions are shown in Supplementary Figure S1.

Table 1. Number of locations and samples of winter wheat grain collected from 2014 to 2017 and in 2019.

Year	Number of Locations	Number of Samples
2014	20	40
2015	20	40
2016	22	44
2017	24	48
2019	25	50
Total	111	222

2.2. Sample Preparation

Pure isolates from five *Fusarium* species (*F. culmorum*, *F. avenaceum*, *F. graminearum* s.s., *F. poae*, and *F. langsethiae*) were cultivated on PDA medium. The surface of the PDA in the Petri dishes was covered with sterilized cellophane disks. Mycelial cultures were incubated in darkness at 22 °C for one week. Between 1 and 2 g of mycelium from each species was harvested for DNA extraction. The samples were ground in liquid nitrogen using a mortar and pestle.

Wheat grain samples (300 g) were finely ground using the Ultra Centrifugal Mill ZM 300 (Retsch Polska, Verder Polska Sp. z o.o., Katowice, Poland). Samples from two wheat cultivars were thoroughly mixed. Then, 5 g of ground grain was powdered in liquid N₂ using the Geno/Grinder 2000 (OPS Diagnostics, Bridgewater, NJ, USA). Two replicates (200 mg) of subsamples were taken from each powdered sample for DNA isolation.

2.3. DNA Isolation

DNA extraction from pure mycelia and plant samples was conducted using a CTAB method. 750 µL of CTAB extraction buffer (20 g/L CTAB, 1.4 M NaCl, 0.1 M Tris-HCl, 20 mM Na₂EDTA) with 20 µL of RNase A (10 mg/mL) was added to each subsample. Buffer volumes, together with the volumes of chemicals used in subsequent steps, were increased proportionally, if needed. Samples were incubated at 65 °C for 15 min. Next, 20 µL of Proteinase K (20 mg/mL) was added, and samples were incubated at 65 °C for 60 min. Subsequently, samples were centrifuged for 10 min at 16,000× g. The supernatant was transferred into a new tube, and 500 µL of chloroform was added. Then, samples were shaken for 30 s and centrifuged for 10 min at 16,000× g. The upper layer was transferred to a new tube and mixed with another 500 µL of chloroform. After 5 min of centrifugation at 16,000× g, the upper layer was combined with two volumes of CTAB precipitation solution (5 g/L CTAB, 0.04 M NaCl). The mix was incubated for 60 min at room temperature and centrifuged for 5 min at 16,000× g. After removing the supernatant, the pellet was dissolved in 350 µL of 1.2 M NaCl. Next, 350 µL of chloroform was added, and the mix was shaken for 30 s. After 10 min centrifugation at 16,000× g, the upper phase was transferred to a new tube and mixed with 180 µL of isopropanol. The sample was centrifuged for 10 min at 16,000× g. The supernatant was removed, and the pellet was washed in 500 µL of 70% ethanol and centrifuged for 10 min at 16,000× g. The DNA pellet was dried and then dissolved in 100 µL of deionized, sterile water. DNA concentration was measured using the Nanophotometer Pearl (Implen GmbH, Munich, Germany).

2.4. Real-Time PCR

Real-time PCR analyses with SYBR Green intercalating dye were performed on an Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Primer sequences developed by Nicolaisen et al. [52] are listed in Table 2. The authors state that *F. graminearum* primers (Fgram F, Fgram R) detect all species in the *F. graminearum* species complex. Exceptions are two species from South America—*F. cortaderiae* and *F. brasiliicum*.

PCR reactions were conducted in a total volume of 12.5 µL. Standard curves for *Fusarium* species were prepared using subsequent dilutions of DNA derived from pure cultures: 0.001, 0.01, 0.1, 1, and 10 ng/µL. The standard curve for the *EF1α* reference gene was prepared from wheat DNA in concentrations of 0.5, 1, 2, 5, and 10 ng/µL. Samples for the real-time PCR reaction were diluted to 5 ng/µL. The PCR mix consisted of 1× SYBR Green PCR Mastermix (Thermo Fisher Scientific, Waltham, MA, USA), 250 nM primer F and R (Sigma-Aldrich, St. Louis, MO, USA), and 0.5 µg/µL BSA (Thermo Fisher Scientific, Waltham, MA, USA). The temperature profile of the PCR reaction was as follows: 50 °C,

2 min; 95 °C, 10 min; (95 °C, 15 s; 62 °C, 1 min) × 40. For the *EF1α* gene, the annealing temperature was 60 °C. Following the PCR reaction, melt curve analysis was conducted in the temperature range of 60–95 °C to check the specificity of the obtained products.

The concentration of each *Fusarium* species was measured using a standard curve. The efficiency values for all *Fusarium* species were approximately $R^2 = 0.99$. The results are expressed as picograms of fungal DNA per microgram of plant DNA, following Nicolaisen et al. [53]. Limits of DNA quantification (LOQ) for specific species were as follows: *F. avenaceum*—100 pg/μg, *F. culmorum*—200 pg/μg, *F. graminearum*—200 pg/μg, *F. langsethiae*—100 pg/μg, and *F. poae*—100 pg/μg.

Table 2. Sequences and names of species-specific primers.

Target	Primer Name	Sequence (5′-3′)
<i>F. graminearum</i> species complex	Fgram F Fgram R	CCATTCCCTGGGCGCT CCTATTGACAGGTGGTTAGTGACTGG
<i>F. culmorum</i>	Fcul F Fcul R	CACCGTCATTGGTATGTTGTCAC CGGGAGCGTCTGATAGTCG
<i>F. langsethiae</i>	Flang F Flang R	CAAGTCGACCACTGTGAGTACCTCT TGTCAAAGCATGTCAGTAAAGATGAC
<i>F. avenaceum</i>	Fave F Fave R	TATGTTGTCACCTGTCTCACACCACC AGAGGGATGTTAGCATGATGAAG
<i>F. poae</i>	Fpoae F Fpoae R	ACCGAATCTCAACTCCGCTTT GTCTGTCAAGCATGTTAGCACAAGT
<i>EF1α</i>	Hor1F Hor2R	TCTCTGGGTTTGAGGGTGAC GGCCCTTGACAGTCAAGGT

2.5. Statistical Analysis

Statistical analysis was performed using Microsoft® Excel 2016 and XLSTAT® (Version 2020.4.1.1027, Addinsoft, Paris, France) software.

Total *Fusarium* DNA over five years, as well as *Fusarium* species DNA in specific years, was analyzed using analysis of variance (ANOVA) procedures with XLSTAT software. One-way (year or species) ANOVAs were performed. Normality of the data distribution was tested using a Shapiro–Wilk test (XLSTAT procedure: Normality test). *Fusarium* DNA amounts did not follow a normal distribution and were transformed with a log₁₀ transformation. Mean differences were determined using Fisher’s LSD test at $p = 0.05$.

The relationships between DNA levels of five *Fusarium* species were analyzed using Pearson’s correlation tests (XLSTAT procedure: Correlation tests). Before analysis, all variables that were not normally distributed were log₁₀ transformed.

The data on DNA levels of five *Fusarium* species in 111 wheat grain samples, collected over five years from 26 locations, were then analyzed using multivariate statistical methods. Principal component analysis (XLSTAT procedure: Principal Component Analysis PCA) was used to demonstrate how grain samples from different locations and years are distributed in relation to the variation explained by the first two principal components and how DNA levels of *Fusarium* species influence these components. PCA results also reveal relationships among variables based on the angles between variable vectors.

To identify a potential link between *Fusarium* DNA levels in grain and the summarized weather variables across 92 phenological periods across sites/years, Spearman’s rank correlation coefficient was used. Weather summaries with a significant Spearman’s correlation ($p < 0.01$) were identified as key weather factors that could influence *Fusarium* colonization of wheat grains. A phenological stage was assigned to these significant weather summaries.

3. Results

3.1. Weather Conditions

The weather conditions at the grain sampling locations varied (Figure 2). The average temperature in May fluctuated over the five years, ranging from 10.2 °C to 16.3 °C. The largest temperature difference between locations was recorded in 2019 and 2015, while the smallest was in 2016. In June, the average temperature ranged from 13.9 °C to 22.8 °C over the five years. The greatest variation between locations was observed in 2015 and 2017, with the smallest in 2014. July's average temperature ranged from 15.9 °C to 22.0 °C during these years, with the highest variation between locations in 2015 and 2017, and the lowest in 2014.

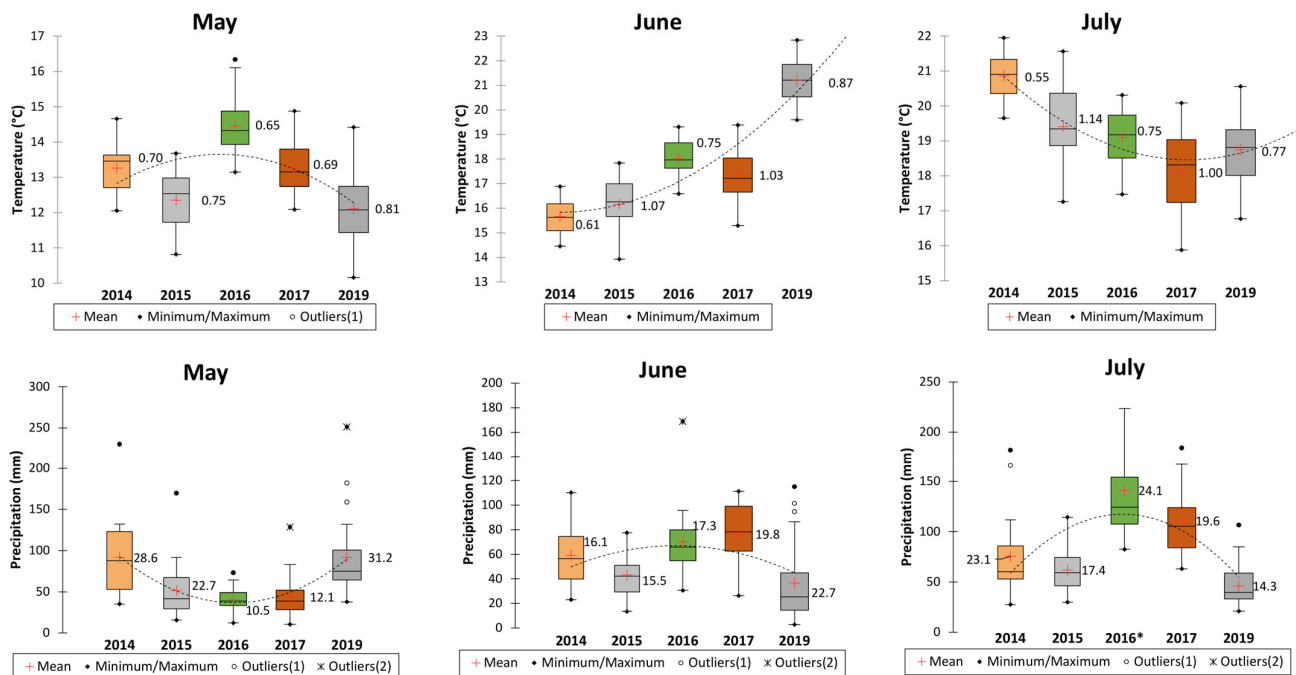


Figure 2. Weather conditions (average monthly temperature and total monthly precipitation) for May, June, and July from 2014 to 2017 and in 2019. Data are averaged from 26 wheat grain sampling locations. Boxes indicate the first quartile, median, and third quartile. Whiskers show the minimum and maximum values without outliers. * The highest total monthly precipitation in July 2016 was 408 mm. Numbers represent the standard deviation calculated without outlier values identified using the Interquartile Range (IQR) method.

Over the five-year period, the lowest average temperatures in May were recorded in 2019 and 2015, while the highest was in 2016. We observed a gradual decrease in average temperature starting in 2016. In June, the lowest average temperature was recorded in 2014 and the highest in 2019. The average temperature in June has increased annually since 2014, reaching a particularly high level in 2019. The lowest average temperature in July occurred in 2017 and 2019, with the highest in 2014. There was a steady decline in the average temperature in July from 2014 onward.

Regarding precipitation, the monthly total rainfall in May ranged from 40.7 to 91.8 mm over five years. The greatest variation between locations occurred in 2019 and 2014, while the least was in 2016. In June, the annual total rainfall ranged from 36.6 to 76.4 mm over a five-year period. The largest variation between locations occurred in 2019, while the smallest was in 2015. In July, the total rainfall over five years varied from 45.9 to 141.2 mm. The highest variation between locations was in 2016 and 2014, and the lowest was in 2019.

Over five years, the lowest precipitation in May was observed in 2016 and 2017, while the highest occurred in 2014 and 2019. We saw a gradual decline in precipitation from 2014 to 2016, followed by an increase until 2019. In June, the lowest precipitation was recorded in 2019 and 2015, and the highest in 2017. The June precipitation remained fairly consistent across the five years. The lowest precipitation in July was recorded in 2019, while the highest was in 2016. We observed a gradual increase in July precipitation from 2014 to 2016, followed by a decrease until 2019.

3.2. *Fusarium* Species

DNA from five *Fusarium* species was detected in most samples over a five-year period. Trace amounts (below LOQ) of *Fusarium* DNA were detected in 40% of samples in 2014, 35% in 2015, 36% in 2016, 8% in 2017, and 28% in 2019 (Table 3).

Table 3. Percentages of wheat grain samples with detected DNA of *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. langsethiae* and *F. poae* and percentage of samples with trace amount of DNA from 2014 to 2017, in 2019 and across years.

Year (Samples)	<i>F. avenaceum</i>	<i>F. culmorum</i>	<i>F. graminearum</i>	<i>F. langsethiae</i>	<i>F. poae</i>	<i>Fusarium</i>
2014 (20)	95/100 *	45/89	100/55	50/90	100/70	100/40
2015 (20)	100/100	55/100	90/89	50/80	100/50	100/35
2016 (22)	100/82	91/60	100/68	91/70	100/68	100/36
2017 (24)	79/74	67/56	100/25	75/67	100/58	100/8
2019 (25)	92/96	88/100	96/79	84/48	96/50	100/28
all years (111)	93/90	70/79	97/62	71/67	99/59	100/29

* Percentage of samples with trace amount of DNA calculated for samples with detected *Fusarium* DNA.

The most frequent species were *F. poae* and *F. graminearum*. They had a similar percentage of samples with trace amounts of DNA. Third species frequently detected was *F. avenaceum*; however, 90% of samples of this species had a trace amount of DNA. *F. culmorum* and *F. langsethiae* were detected less frequently and more samples of with *F. culmorum* had only trace amounts of DNA.

The highest total amount of *Fusarium* DNA was identified in 2017, and it was notably higher than in other years (Figure 3). In 2014, *F. graminearum* s.s. dominated, and the amount of DNA from this species was significantly higher than that of the other species. Only traces of *F. avenaceum* DNA were detected. In 2015, *F. poae* was the dominant species, and its DNA amount was greater than that of the others. DNA from *F. avenaceum* and *F. culmorum* was detected only in trace amounts. In 2016, the DNA levels of the five species did not differ significantly; however, the highest amounts were seen for *F. avenaceum* and *F. graminearum* s.s., while *F. langsethiae* had the lowest amount. In 2017, *F. graminearum* s.s. again dominated, and its DNA amount was significantly higher than that of the other species. In 2019, the DNA levels of the five species did not differ significantly, with *F. avenaceum* having the lowest amount. DNA from *F. culmorum* was detected in trace amounts.

The highest concentrations of total *Fusarium* DNA during the five-year study were found in southeastern Poland, especially in the Podkarpackie province, as well as in the northwestern regions, including Zachodniopomorskie and Pomorskie provinces (Figure 4). Elevated levels of *Fusarium* DNA were also detected in wheat grain from the southern Opolskie province. However, it is important to note that grain samples from this province were only available from 2017, when the DNA *Fusarium* levels were high. Additionally, the Małopolskie province in the south showed higher concentrations of *Fusarium* DNA compared to other regions. The lowest levels were observed in Kujawsko-Pomorskie (north-central Poland), Podlaskie (eastern Poland), and Dolnośląskie (southwestern Poland).

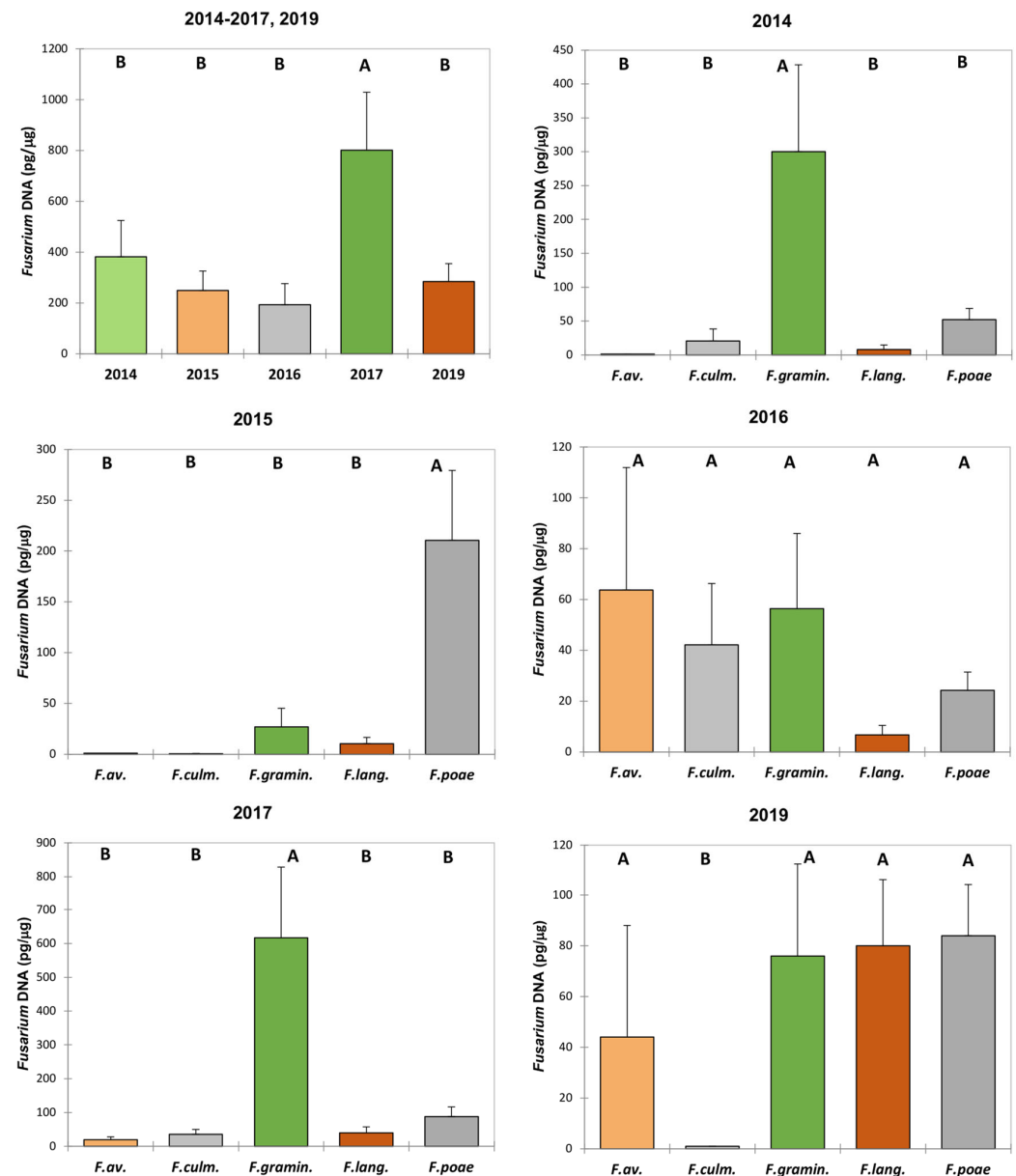


Figure 3. Average DNA concentration (pg/mg of wheat DNA) for five *Fusarium* species in winter wheat grain samples from 2014 to 2017 and 2019. 2014–2017, 2019—total DNA concentration of the five species each year. F.av.—*F. avenaceum*; F.culm.—*F. culmorum*; F.gramin.—*F. graminearum* s.s.; F.lang.—*F. langsethiae*. The number of sampling locations is indicated (two grain samples per location). Means marked with the same letter are not significantly different at $p < 0.05$ according to Fisher's LSD test.

Over five years of experimentation, the regional distribution of *Fusarium* DNA showed significant variation (Figure S1). In 2014, *Fusarium* DNA was mainly found in grain samples from southeastern Poland (Podkarpackie, Lubelskie, and Małopolskie provinces), with particularly high levels in Podkarpackie (2804 pg/μg). In 2015, a large amount of *Fusarium* DNA was detected in samples from southern Poland, especially in Małopolskie. Unlike in 2014, high concentrations also appeared in samples from Mazowieckie. In 2016, *Fusarium* DNA was primarily found in samples from northwestern Poland (Zachodniopomorskie), with a concentration of 1757 pg/mg, while other regions showed lower levels. An exception was Śląskie in southern Poland, which exceeded the average for 2016 at 501 pg/mg (Figure 3). In 2017, the highest *Fusarium* DNA levels were in samples from northwest and north Poland (Zachodniopomorskie, Pomorskie, and Warmińsko-Mazurskie). Lev-

els were also high in parts of central Poland (Wielkopolskie and Łódzkie) and southern Poland (Opolskie). Unfortunately, samples from Podkarpackie were not available this year. In 2019, *Fusarium* DNA was mainly found in grain samples from northern (Pomorskie and Warmińsko-Mazurskie) and southeastern Poland (Podkarpackie, Małopolskie, and Świętokrzyskie), with high levels also in western Poland (Lubuskie).

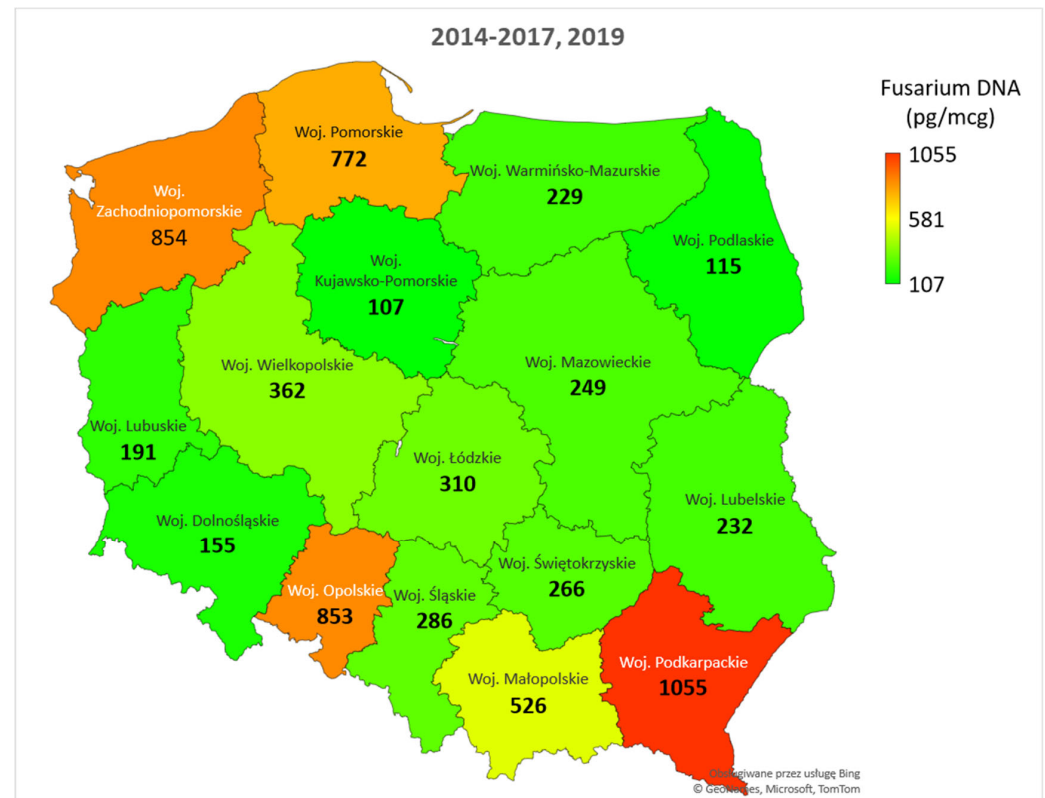


Figure 4. Regional (provincial) averages of total DNA concentration (pg/μg of wheat DNA) for five *Fusarium* species in winter wheat grain samples collected from 2014 to 2017 and in 2019. Woj.—province. In some regions, there are no grain samples from certain years. See Figure S1.

We analyzed the frequency of *Fusarium* species in Polish regions over five years (Figure S2). DNA from *F. avenaceum* was found in significant amounts only in two northern regions—the Zachodniopomorskie and Pomorskie provinces. In other regions, it was present in very low or trace amounts. The DNA concentration of *F. culmorum* in wheat grain was generally low, mainly detected in samples from the northwestern Zachodniopomorskie province and the southern Śląskie province. Only traces of *F. culmorum* DNA were found in most parts of eastern Poland, as well as in the south and west. DNA from *F. graminearum* s.s. was detected in the highest amounts, mostly in southeastern and southern Poland (particularly in the Podkarpackie province), as well as in northern and northwestern Poland (mainly Pomorskie). It was also present in central and west-central regions. Only trace levels of this species' DNA were found in the Mazowieckie and Lubuskie provinces. The presence of *F. langsethiae* DNA was confirmed in significant quantities in two southern regions (Opolskie and Podkarpackie) and one in western Poland (Lubuskie). In five of the sixteen provinces, *F. langsethiae* was either not detected or found only in trace amounts. The DNA of *F. poae* was detected in most provinces, with the highest concentrations in the southern Małopolskie province and the east-central Mazowieckie province. Only low or trace amounts of this species' DNA were observed in two regions.

The DNA amounts of three *Fusarium* species—*F. avenaceum*, *F. culmorum*, and *F. graminearum* s.s.—were significantly correlated (Table 4). The highest correlation was

observed between *F. avenaceum* and *F. culmorum*. In samples with high levels of *Fusarium* DNA, these two species were often found together (Figure S3). The correlation coefficients between *F. graminearum* and either *F. avenaceum* or *F. culmorum* were significant but low. *F. graminearum* s.s. was present in fewer samples, along with one of these species, in higher amounts. However, some grain samples were infected equally by all three species mainly in 2017 and 2016 (Figure 5). No correlation was found between the DNA of *F. langsethiae* or *F. poae* and that of the other species.

Table 4. Pearson correlation matrix of DNA concentrations from five *Fusarium* species.

Variables (n = 111)	<i>F. avenaceum</i>	<i>F. culmorum</i>	<i>F. graminearum</i> s.s.	<i>F. langsethiae</i>
<i>F. culmorum</i>	0.441			
<i>p</i> -value	0.000			
<i>F. graminearum</i> s.s.	0.190	0.196		
<i>p</i> -value	0.046	0.040		
<i>F. langsethiae</i>	0.029	0.088	0.055	
<i>p</i> -value	0.766	0.360	0.570	
<i>F. poae</i>	−0.040	0.042	0.024	−0.025
<i>p</i> -value	0.675	0.659	0.800	0.794

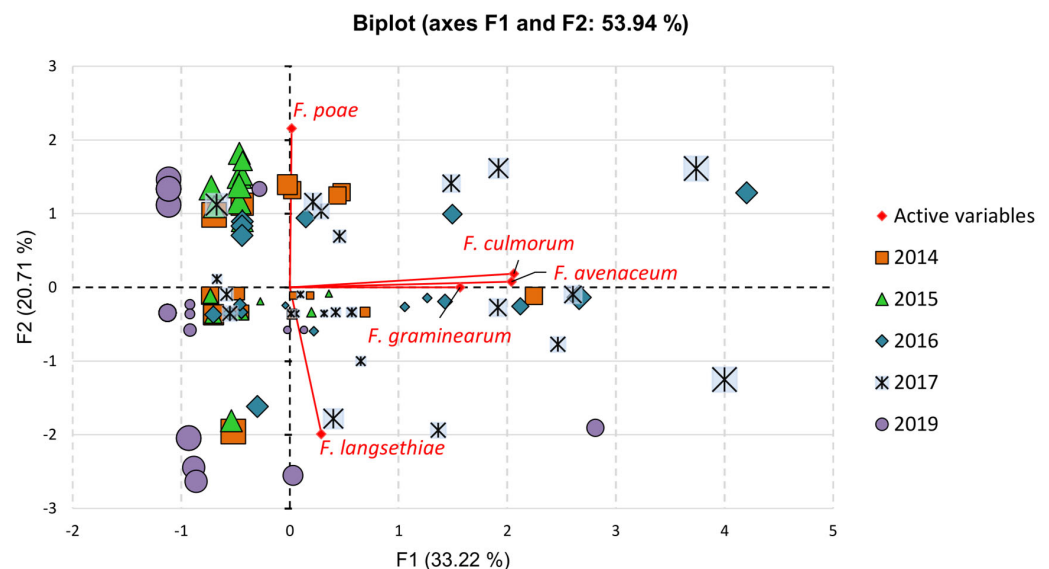


Figure 5. Biplot of the principal component analysis (PCA) of the DNA concentration (pg/mg) of five *Fusarium* species in samples of winter wheat grain collected over five years (2014–2017, 2019). The factor loading values based on the correlations between the factors (F1 and F2, respectively) and *F. avenaceum*, *F. culmorum*, *F. graminearum* s.s., *F. langsethiae*, and *F. poae* are equal: 0.794 and 0.027, 0.803 and 0.064, 0.610 and 0.001, 0.113 and −0.688, and 0.006 and 0.764, respectively. The size of the points shows the sum of squared cosines on the first two factors.

The relationships between species were further analyzed using Principal Component Analysis (PCA). A strong connection was found among the DNA concentrations of *F. avenaceum*, *F. culmorum*, and *F. graminearum* s.s. (Figure 5). All three species showed high positive loadings on factor F1. These species were often detected together in the same grain samples. High DNA levels were mainly observed in samples from 2017 and 2016, with some notable amounts also found in samples from 2014 and 2019. No significant link was observed between these species and *F. langsethiae* or *F. poae*. For factor F2, the loadings were highly positive for *F. poae* and highly negative for *F. langsethiae*. *F. langsethiae* DNA was only detected in larger amounts in certain samples, mainly from 2016, 2017, and 2019. *F. poae* was consistently found in many samples across all years, but in similar moderate quantities.

The loadings for factor F3 showed that in some samples—mainly from 2019—both species were present in significant amounts (Figure S4). Again, there was no relationship between these species and *F. avenaceum*, *F. culmorum*, or *F. graminearum* s.s.

Factor loadings for factor F4 were strongly positive for *F. graminearum* and slightly negative for the other species (Figure S5). This indicates the dominance of *F. graminearum* as a key pathogen in the FHB complex of wheat.

The relationships between two weather variables (daily mean temperature, precipitation), estimated over 14-day windows during the growing season, and the total *Fusarium* DNA content in winter wheat grain at harvest were analyzed using Spearman's rank correlation coefficient (Figure 6). A positive correlation was observed between DNA content and precipitation during flowering (BBCH 61–69) and ripening (BBCH 87–89) ($r = 0.20$ – 0.23) and ripening (BBCH 87–89) ($r = 0.33$). Conversely, daily mean temperature during stem elongation (BBCH 30–39) and milk-dough (BBCH 75–83) development was negatively correlated with DNA content in the harvested grain ($r = -0.21$ – -0.34). Daily mean temperature was positively correlated with DNA content at booting-heading (BBCH 45–51) ($r = -0.21$ – -0.36).

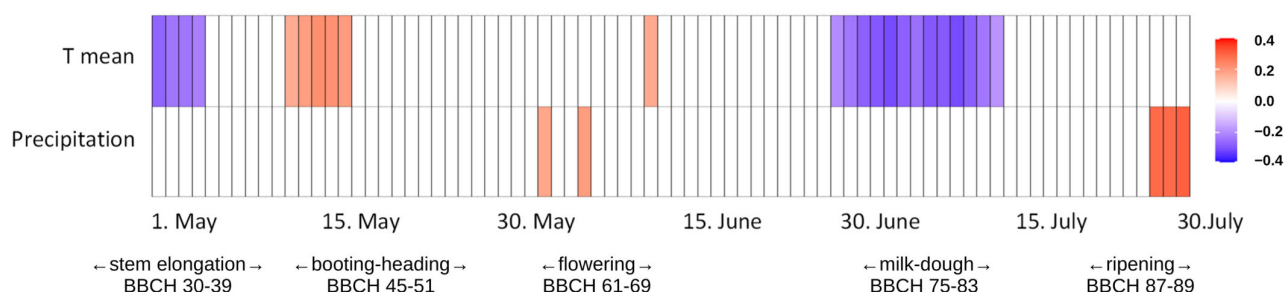


Figure 6. Spearman's rank correlation coefficient for *Fusarium* DNA content in winter wheat at harvest and different weather factors estimated for 14-day moving windows during the growing season. Red indicates a positive correlation and blue a negative correlation (both $p \leq 0.01$) between DNA content and a particular weather variable, with a darker color indicating a higher value of the correlation coefficient. Tmean = daily mean temperature.

4. Discussion

This study emphasizes the significant impact of weather conditions on the presence and distribution of *Fusarium* species in winter wheat grain. The yearly variations in average temperatures during May, June, and July—especially the extreme differences observed in 2019 and 2014—along with the more stable conditions in 2017, demonstrate that temperature is a key factor in *Fusarium* development. This is further supported by the negative correlation between temperature during the stem elongation and milk-dough stages and *Fusarium* DNA content. Previous research has also highlighted the role of temperature in shaping the dynamics of *Fusarium* species [53–55].

Precipitation levels exhibited considerable annual variation, with notable differences between the dry year of 2015 and the wet years of 2016 and 2017. The positive correlation between *Fusarium* DNA content and precipitation during the flowering and ripening stages suggests that increased moisture may promote the growth and spread of *Fusarium* species. This supports previous research emphasizing the significance of wet conditions in *Fusarium* development [56–65].

The detection of *Fusarium* DNA in most samples collected in the late 2010s, with the highest concentrations in southeastern and northwestern Poland, indicates that regional climate factors influence the prevalence of *Fusarium*. We found that *F. graminearum* s.s. was the primary cause of FHB. The proportion of the previously dominant species, *F. culmorum*, decreased significantly. It was found in higher amounts only in the cooler northwestern region. Duba et al. [59], investigating wheat samples from northeastern and southeastern

Poland (collected in 2014 and 2015), found *F. culmorum* dominance only in grain from the cooler northern region.

Several studies examining changes in *Fusarium* populations, mainly in Western Europe, have shown similar trends. According to Birzele et al. [60] in 1997 and 1998, the dominant species in wheat grain in Germany were *F. avenaceum*, *F. poae*, *F. culmorum*, and *F. graminearum*. The frequencies of the last two species were similar; however, the percentage of *F. graminearum* increased in 1998. Waalwijk et al. [61] analyzed wheat heads and grain collected in the Netherlands in 2001 and 2002. In 2001, samples collected during the late milk stage mainly contained *F. graminearum*; however, some samples also had *F. avenaceum* and/or *F. culmorum*. At harvest, *F. graminearum* was almost exclusively dominant. In 2002, weather conditions were more favorable for FHB, leading to a relative dominance of *F. graminearum* in grain from the Netherlands and nearly complete dominance in samples from France. Isebaert et al. [62] observed that *F. graminearum* and *F. culmorum* were the most prevalent species in Northern Belgium from 2002 to 2005. They found an interesting correlation between crops and the frequency of both species (maize—*F. graminearum*, small grain cereals—*F. culmorum*). In Luxembourg, the most common species found on wheat heads in 2008 were *F. graminearum*, *F. avenaceum*, and *F. poae*. An increase in the frequency of *F. graminearum* and a decrease in *F. culmorum* were observed [63].

Chandelier et al. [64] analyzed winter wheat samples from Belgium over the 2003–2009 period. They found that the main species were *F. avenaceum* and *F. graminearum*; however, their frequency varied depending on the year, ranging from 20% to 100%. The frequency of *F. poae* remained relatively constant over the years (about 70%). The overall incidence of *F. culmorum* decreased during the study, from 80% in 2003 to 10% in the final three years. In Germany in 2008, *F. graminearum* s.s. was the main species, followed by *F. culmorum*. Other species (*F. poae*, *F. tricinctum*, *M. nivale*, etc.) were found in small quantities [16]. In Hungary, in 2010, which was a very favorable year for FHB development, *F. graminearum* was primarily isolated from wheat grain [65]. Winter wheat grown in the Netherlands in 2009 was examined for the presence of *Fusarium* species and toxins [66]. In samples collected at harvest, the authors observed a dominance of *F. graminearum*. *F. avenaceum* and *Microdochium nivale* were also common. However, in the preharvest samples, only *F. graminearum* and *M. nivale* were found.

In a survey of winter wheat in Northern Germany from 2013 to 2017, Birr et al. [23] found that *F. graminearum* was dominant. Other species, such as *F. culmorum*, *F. avenaceum*, and *F. poae*, were less common but still made up a significant part of the FHB complex. *F. langsethiae* was also detected, though at lower frequencies. During dry years from 2014 to 2016, *Fusarium* DNA levels were lower, and the proportions of the four main species were nearly the same. The authors did not observe dominance of species other than *F. graminearum*, such as *F. poae*, in 2015 in this study, and they detected a much higher amount of *F. culmorum* DNA than shown here.

Fusarium poae was the most frequently detected species in the grain, appearing in 88% of samples, compared to 82% for *F. graminearum*. Other species were less common: *F. avenaceum* in 78% of samples, *F. langsethiae* in 67%, and *F. culmorum* in 58%. The amount of *F. poae* DNA was approximately three times lower than that of *F. graminearum* over a five-year period. However, during dry years like 2015, *F. poae* dominated, with its DNA levels being eight times higher than *F. graminearum*. In similarly dry conditions in 2019, the DNA amounts for both species were similar.

According to published studies, *F. poae* was frequently isolated from wheat spikes and kernels in Poland in 1993, as well as in 2006–2007 [67,68]. It is a weak pathogen of cereal spikes; however, it is widespread on wheat across Europe [7,62,69,70]. In 2001 and 2002, Xu et al. [69] discovered that *F. poae* was the most common species infecting grain at harvest

in three studied countries (UK, Italy, Hungary). *Fusarium poae* was less common only in cooler Ireland. Audenaert et al. [71] observed that this species was dominant in Flanders in 2007, although it was isolated less frequently in 2008. The higher infection pressure in 2007 compared to 2008 was attributed to *F. poae*'s nature as a secondary pathogen. In Poland, Lenc et al. found *F. poae* to be the most common species on spring wheat grain during 2011–2013 [72]. The other species, including *F. graminearum* and *F. culmorum*, were less frequent.

Additionally, the frequent occurrence of *F. poae* is related to its sporulation strategy. This species produces large amounts of tiny microconidia in a dry, powdery form that can easily infect cereal heads. This process may be particularly effective in dry conditions and wind dispersal, as Hörberg [73] found no difference between the dispersal patterns of *F. poae* microconidia and the much larger macroconidia of *F. culmorum* during splash dispersal. Xu et al. [74] linked *F. poae* with relatively dry and warm conditions, while *F. graminearum* was associated with warm and humid environments. *F. avenaceum* and *F. culmorum* were both linked to cooler, wet, and humid environments. Parikka et al. [28], who predicted an increase in the importance of *F. poae* (along with *F. langsethiae*) under drier conditions in Scandinavia, also confirmed this. Valverde-Bogantes et al. [21] reviewed the replacement of *F. graminearum* by *F. poae* in European countries, as well as in Canada and Argentina. Most cited authors linked *F. poae*'s dominance with dry conditions in certain years.

Fusarium poae is known as a producer of NIV [75,76]. In Poland, NIV was primarily found in oat grain infected by *F. poae*, but also in wheat grain in FHB epidemic years [77,78]. Other species, such as *F. graminearum* and *F. culmorum*, are also involved in NIV production [79]. However, NIV chemotypes of these species are not common in Poland [37]. Stępień et al. [80] reported that only 12% of *F. graminearum* isolates in Poland displayed the NIV chemotype. *F. poae* was also found to produce a wide range of toxins, including trichothecenes of types A and B, beauvericin, enniatins, moniliformin, and others [5,75,81,82].

The strong correlation among *F. avenaceum*, *F. culmorum*, and *F. graminearum* s.s. suggests that they may share ecological niches or respond to similar environmental triggers. The PCA supports these findings, indicating that these species often coexist, especially in samples from 2017 and 2016. Similar correlations were observed by Birr et al. [23] in Northern Germany from 2013 to 2017. The highest correlation coefficient was between *F. avenaceum* and *F. culmorum*. They also observed a high coefficient for the *F. culmorum*–*F. graminearum* relationship. However, compared to this research, they detected higher amounts of *F. culmorum* DNA. The lack of correlation with *F. langsethiae* or *F. poae* suggests that these species may respond to different environmental factor conditions. Xu et al. [69] stated that when weather conditions are unfavorable (e.g., drought) for *F. graminearum*, it can be replaced by secondary FHB species, such as *F. poae*, which was the most common. The negative correlation between temperature during the stem elongation and milk-dough stages and *Fusarium* DNA content suggests that higher temperatures at these stages may not promote *Fusarium* infection, possibly due to faster plant growth or changes in plant susceptibility. Conversely, the positive correlation during booting and heading suggests that the temperature at this stage could lead to increased infection rates.

Overall, this study emphasizes the complex interactions between weather conditions and the presence of *Fusarium* species in winter wheat grain. Understanding these relationships is essential for developing effective management strategies to reduce the impact of *Fusarium* on wheat production. The composition of FHB-causing *Fusarium* species is key to understanding mycotoxin profiles in wheat grain, as was shown in many published studies [8,83–85]. Future research should focus on exploring how temperature and precipitation specifically influence *Fusarium* growth and identifying potential methods to lower *Fusarium* and mycotoxin contamination in wheat.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15102430/s1>, Figure S1: Regional (provincial) averages of the total DNA concentration (pg/ μ g of wheat DNA) of five *Fusarium* species in winter wheat grain samples collected from 2014 to 2017 and 2019. Data are shown for specific years and the five-year average. For province names, refer to Figure 4. Figure S2: Regional (provincial) averages of the DNA concentration (pg/ μ g of wheat DNA) of five *Fusarium* species in the grain of winter wheat samples collected from 2014 to 2017 and 2019. For province names, refer to Figure 4. Figure S3: Scatter plots of pairwise correlations between log10-transformed DNA quantities of *Fusarium graminearum* and other *Fusarium* species (*F. avenaceum*, *F. culmorum*, *F. langsethiae*, *F. poae*), as well as between log10-transformed DNA quantities of *F. avenaceum* and *F. culmorum*, including Pearson correlation coefficients (*r*) and *p*-values. Figure S4: Biplot of the principal component analysis (PCA) of DNA concentration (pg/mg) for five *Fusarium* species in 111 winter wheat grain samples collected over five years (2014–2017, 2019). Factor loading values based on the correlations between Factors (F1 and F3, respectively) and *F. avenaceum*, *F. culmorum*, *F. graminearum* s.s., *F. langsethiae*, and *F. poae* are 0.794 and -0.249 , 0.803 and -0.026 , 0.610 and 0.225, 0.113 and 0.628, and 0.006 and 0.640, respectively. The size of the points reflects the sum of the squared cosines on Factors 1 and 3. Figure S5: Biplot of the principal component analysis (PCA) of DNA concentration (pg/ μ g) for five *Fusarium* species in 111 winter wheat grain samples collected over five years (2014–2017, 2019). The factor loading values based on correlations between Factors (F1 and F4, respectively) and *F. avenaceum*, *F. culmorum*, *F. graminearum* s.s., *F. langsethiae*, and *F. poae* are 0.794 and -0.228 , 0.803 and -0.321 , 0.610 and 0.759, 0.113 and -0.203 , and 0.006 and -0.152 , respectively. The size of the points reflects the sum of the squared cosines on Factors 1 and 4.

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