

CHARACTERIZATION OF BLACK POINT DISEASE IN WINTER WHEAT: EPIDEMIOLOGY, SEVERITY SCORING AND FUNGAL COMMUNITY STRUCTURE

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Abstract

Black point is a common fungal disease of wheat caused by several genera, including Alternaria sp., Cochliobolus sp., Cladosporium sp., and Fusarium sp., leading to embryo discoloration and reduced grain quality. This study evaluated winter wheat varieties to characterize disease epidemiology, assess the incidence and severity of kernel darkening, and identify the associated fungal community. Favorable weather conditions during grain filling resulted in variable disease levels among genotypes. Significant differences were observed in both the proportion of affected kernels and the severity of discoloration. Fungal isolations confirmed Alternaria sp. as the dominant pathogen, accompanied by Cochliobolus sp., Cladosporium sp., Fusarium sp., Epicoccum sp., Curvularia sp., and Chaetomium sp.. The results provide insights into varietal responses to black point under Romanian conditions and support breeding and management strategies to mitigate disease impact and maintain grain quality.

Key words: winter wheat, black point, kernel discoloration, fungal disease.

INTRODUCTION

Winter wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide. Wheat represents a fundamental component in ensuring global food security (Sharma & Sharma, 2025).

In Romania, autumn wheat is the most widely cultivated crop due to its high economic importance and the favorable environmental conditions for its cultivation. In 2024, approximately 9.3 million tons of wheat were harvested from an area of about 2.3 million hectares (FAO, 2025).

The quality of wheat kernels is a key factor in determining the market value of the crop and in appropriately assessing its suitability for processing, particularly in the milling and baking industries (Awulachew, 2020). However, wheat production is often affected by various diseases, especially fungal ones, which compromise both yield and kernel quality (Duveiller et al., 2007; Aakash Goyal et al., 2010; Rózewicz et al., 2021).

Among the fungal and physiological factors that influence wheat kernel quality, black point is a significant defect (Malaker et al., 2009;

Srivastava et al., 2014). This disorder, manifested by discoloration in the embryo region of the kernels, can considerably reduce both the visual appearance and the technological value of wheat, especially when it is intended for the milling and baking industries (Clarke et al., 2005; Golosna, 2021). Some countries have established limits for the percentage of wheat kernels affected by black point. Globally, these limits vary significantly, as there is no international standard that clearly defines this disorder, and regulation is primarily carried out through national standards. For example, in Australia, the competent authorities have set a maximum limit of 5% for bread wheat and 3% for durum wheat. Exceeding these thresholds results in the classification of wheat lots as downgraded (Rees et al., 1984; Lehmensiek et al., 2004). In Canada, according to the Canadian Grain Commission, black point is a criteria used to classify wheat kernels into different grades. For durum wheat, the tolerance levels are 5%, 10%, and 20% for Grades No.1, No.2, and No.3, respectively, while for common wheat, the tolerances are 10%, 20%, and 35% for the same grades (Canadian Grain Commission, 2025). Another country that has established wheat

kernel quality standards based on black point symptoms is Turkey (Aktaş and Endes, 2025). According to Turkish Standards Institution standards, wheat is considered of low quality if more than 8% of the kernels are affected. The maximum threshold for purchase is 14%, highlighting the restrictions imposed by many countries in international trade (TSE, 2023). In Romania, national standards, aligned with European regulations, do not specify percentage limits exclusively for black point, unlike in the countries mentioned above.

For common wheat, the grading manual includes a factor called “kernels with discolored testa,” without an explicit reference to black point, with a maximum limit of 0.5% for Grades 1 and 2; Grade 3 is not regulated.

For durum wheat, the grading factor “stained kernels” sets maximum limits of 2% for Grade 1, 3% for Grade 2, and 5.8% for Grade 3 (CNGSC, 2017). Given the significant impact of black point on wheat kernel quality, understanding the occurrence of this disease, its

severity, and the associated fungal communities is essential.

This study aims to characterize black point in winter wheat by evaluating the disease’s epidemiology, determining its incidence in the studied kernels using severity scoring, and investigating the structure of the associated fungal communities.

MATERIALS AND METHODS

The winter wheat kernels used in this study were harvested from the wheat experimental platform organized by ACCPT Iași “Grânarii” at the S.C. Agralmixt S.A. farm during the 2024–2025 agricultural year.

The experimental platform was established in Andrieșeni, Iași County (approximate coordinates: 47°29’50”N, 27°16’50”E).

The area is characterized by a temperate continental climate, with moderate precipitation and mean annual temperatures of about 11.8°C (Table 1).

Table 1. Monthly climatic conditions and wheat phenophases during the 2024–2025 growing season

Month	Air temperature (°C)			Precipitation	Phenophase	Average Air temperature(°C)	Precipitation (mm)
	Minimum	Maximum	Average				
September	5.55	41.12	19.86	131.0	Field preparation Sowing Emergence	11.25	214.6
October	-3.81	33.77	10.86	37.0			
November	-7.14	24.92	3.05	46.6			
December	-8.56	17.45	1.79	56.6	Winter reserve	2.69	112.0
January	-9.63	20.98	2.55	13.4			
February	-14.59	15.07	-2.13	5.0			
March	-6.41	28.07	8.55	37.0			
April	-4.74	35.56	12.09	24.8	Booting – Heading – Flowering	13.19	183.2
May	0.45	37.34	14.28	158.4			
June	6.59	39.37	22.75	18.8	Grain filling	22.75	18.8
July	9.30	43.66	24.99	98.2	Full maturity	24.00	98.2
August	3.49	42.21	23.45	5.4	Post-harvest	23.45	5.4
Average (°C) / Sum (mm)		-2.46	31.63	11.84	632.20		

Note: Climatic data were obtained from the weather station 00001CED located within the experimental farm, via the website www.fieldclimate.com

The climatic conditions during the 2024-2025 growing season created favorable environments for the development of black point disease. Consequently, kernels harvested from the 34 wheat cultivars (Table 2) were analyzed for the presence of black point symptoms.

Regarding cultivation practices, a uniform agronomic protocol was applied across all 34 cultivars. Sowing was performed in the autumn

of 2024, during the optimal period for the region (24-25 October 2024). The preceding crop was sunflower. Fertilization was standardized, with 200 kg of NPK 14:28:14 + 5.5 S applied per hectare. Three phytosanitary treatments were applied to control all winter wheat-specific pests, weeds, and pathogens. The crop was harvested on 22 July 2025.

Table 2. Winter wheat cultivars included in the study

No.	Winter wheat cultivar	No.	Winter wheat cultivar
1	ADRIATIC	18	GLIA
2	AGENOR	19	JUNIOR
3	ANAPURNA	20	LEONIDUS
4	APEXUS	21	MIX CEREALE
5	APILCO	22	MUSIK
6	ARTIMUS	23	PASSION
7	ASTERION	24	PG 102
8	AVENUE	25	PIBRAC
9	BASILIO	26	PROMITOR
10	BOROMIR	27	ROCAILLOU
11	CELEBRITY	28	RUBISKO
12	CHEVIGNON	29	SACRAMENTO
13	COMPLICE	30	SPHERE
14	DATINA	31	STK 3
15	EXTREME	32	STK EXP
16	FALADO	33	TOSCADOU
17	FARMEO	34	ULTIM

The evaluation of black point infection was performed on wheat kernels obtained from the studied wheat cultivars. To determine the incidence of black point infection, 100 caryopses per cultivar were analyzed in three replications. The wheat kernels were randomly selected using a sample divider. The caryopses were carefully examined (Figure 1), and the incidence (%) was calculated as the percentage of affected kernels (grade ≥ 1) out of the total number of analyzed kernels.

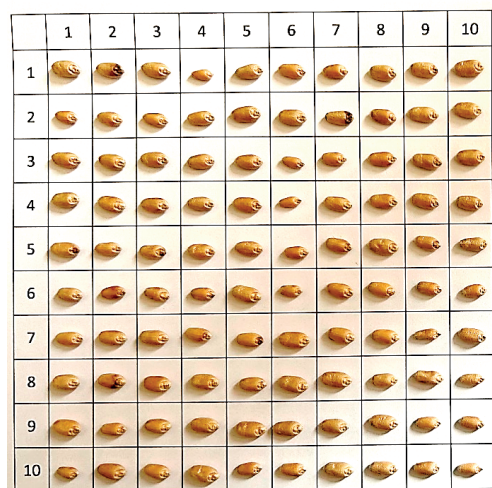


Figure 1. Arrangement of 100 wheat kernels (10×10 grid) used for the visual evaluation of black point incidence. Each kernel was individually examined and classified according to the presence of symptoms

Disease severity was assessed using a six grades rating scale (0-5) adapted for black point in wheat (Table 3), according to the visual

symptoms observed on the kernels. (Gilchrist, 1985).

Tabel 3. Rating scale for black point severity

Rating scale	
Grade 0	Kernel free from any discoloration (apparently healthy)
Grade 1	Tip of the embryo brown to blackish
Grade 2	Discoloration covering the whole embryo
Grade 3	Embryo with $\frac{1}{4}$ of the grain discoloured
Grade 4	Embryo with $\frac{1}{2}$ of the grain discoloured
Grade 5	Embryo with more than $\frac{1}{2}$ of the grain discoloured and shrivelled

A total of 100 kernels per sample were evaluated and classified into the corresponding severity classes. The percentage of kernels in each class was then used to calculate the Disease Severity Index (DSI) as follows (Mishra et al., 2017):

$$DSI(\%) = \left[\frac{\sum(s*n)}{N*S_{max}} \right] * 100,$$

where:

s represents the severity class (0-5);

n is the number of kernels in each class;

N is the total number of analysed kernels;

S_{max} is the maximum score of the rating scale.

To determine the fungal communities associated with wheat kernels affected by black point, 100 kernels from each of the 34 studied cultivars were analysed, of which 75 exhibited visible black point symptoms and 25 were apparently healthy, randomly selected from the harvested samples. The kernels were disinfected by immersion in a 1% sodium hypochlorite solution for 3 minutes, followed by 3–4 rinses with sterile distilled water to remove any residual disinfectant. After drying, the kernels were placed on Petri dishes (9 cm diameter) containing 20 ml of PDA medium (Potato Dextrose Agar) and incubated at 28°C for 5–8 days. Subsequently, emerging fungal colonies were observed and their presence was recorded per plate. Colonies were identified based on their morphological characteristics (Ellis and Ellis, 1985). The aim of this study was to assess the presence of fungi rather than to perform a numeric quantification for each individual kernel.

Statistical analysis of the data was performed using IBM SPSS Statistics 26 software.

Differences among cultivars were evaluated by one-way analysis of variance (ANOVA). Mean comparisons and the identification of homogeneous groups of cultivars were conducted using Tukey's HSD (Honestly Significant Difference) test at a significance level of $p \leq 0.05$. Mean values followed by different letters indicate statistically significant differences among cultivars.

RESULTS AND DISCUSSIONS

Black point was observed in wheat kernels across the studied cultivars, with incidence and severity influenced by environmental conditions during the early grain-filling stage (Conner et al., 1998). Factors such as high humidity, prolonged rainfall, and extreme temperatures are known to promote fungal colonization and accelerate disease progression (Khani et al., 2018). The disease is typically associated with fungal species including *Alternaria* sp., *Fusarium* sp., *Epicoccum* sp., and *Bipolaris* sp., which can colonize the kernels either before or shortly after anthesis (Nuray, 2004; Toklu et al., 2008; Gafencu et al., 2019). Epidemiological studies indicate that both host susceptibility and the timing of environmental stress play crucial roles in determining the extent of infection. High moisture conditions favour spore germination and fungal growth, while temperature fluctuations can further exacerbate the susceptibility of developing kernels.

Under the climatic conditions and using the biological material studied during the 2024-2025 growing season, the following results regarding the presence of winter wheat kernels exhibiting symptoms associated with black point are presented in Table 4.

Symptoms associated with black point disease were observed in the wheat kernels of all cultivated varieties, except for the Sacramento cultivar. The disease occurred at varying intensities across the varieties, highlighting differences in susceptibility among the cultivars examined. The frequency (%) of black point infection ranged from 0.00% in Sacramento to 12.00% in Datina, indicating a significant variation in the proportion of infected kernels among cultivars. The lowest frequency values were also observed in Rubisko (0.67%±0.58%) and Junior (0.67%±0.58%), suggesting that

these varieties exhibited higher tolerance under the experimental conditions. In contrast, the varieties Datina (12.00%±2.00%), Avenue (11.33%±1.53%), and Asterion (11.00%±1.00%) showed the highest frequency values, indicating greater susceptibility to black point occurrence. Intermediate frequency values (approximately 5-7%) were observed in Adriatic, Anapurna, Apexus, and Boromir, indicating moderate susceptibility.

Tabel 4. Frequency and Disease Severity Index of black point in wheat kernels across studied cultivars

Winter wheat cultivar	Frequency (%)	Disease Severity Index (DSI%)
ADRIATIC	5.33% ± 1.53% ^c	1.73% ± 0.42% ^b
AGENOR	6.67% ± 1.53% ^{de}	2.93% ± 0.90% ^h
ANAPURNA	5.67% ± 1.53% ^{cd}	2.40% ± 0.69% ^f
APEXUS	6.67% ± 0.58% ^{de}	2.40% ± 0.60% ^f
APILCO	3.67% ± 1.53% ^{ab}	1.07% ± 0.61% ^c
ARTIMUS	4.33% ± 1.15% ^{bc}	1.07% ± 0.31% ^c
ASTERION	11.00% ± 1.00% ^g	4.80% ± 0.35% ^g
AVENUE	11.33% ± 1.53% ^g	5.47% ± 1.10% ^k
BASILIO	2.00% ± 1.00% ^a	0.73% ± 0.42% ^d
BOROMIR	10.67% ± 2.52% ^{fg}	4.60% ± 0.69% ^g
CELEBRITY	5.33% ± 0.58% ^c	2.67% ± 0.42% ^e
CHEVIGNON	4.33% ± 2.08% ^{bc}	1.13% ± 0.61% ^c
COMPLICE	1.33% ± 1.53% ^a	0.27% ± 0.31% ^a
DATINA	12.00% ± 2.00% ^g	5.53% ± 0.64% ^k
EXTREME	8.00% ± 1.00% ^{ef}	3.53% ± 0.95% ^j
FALADO	3.00% ± 1.00% ^{ab}	1.07% ± 0.31% ^c
FARMEO	1.33% ± 0.58% ^a	0.33% ± 0.12% ^a
GLIA	2.67% ± 0.58% ^{ab}	1.07% ± 0.61% ^c
JUNIOR	0.67% ± 0.58% ^a	0.40% ± 0.40% ^b
LEONIDUS	4.67% ± 0.58% ^{cd}	2.27% ± 0.42% ^d
MIX CEREALE	6.67% ± 0.58% ^{de}	2.87% ± 0.31% ^e
MUSIK	2.33% ± 1.15% ^{ab}	0.87% ± 0.61% ^c
PASSION	2.67% ± 0.58% ^{ab}	0.73% ± 0.31% ^d
PG 102	5.00% ± 1.00% ^c	1.93% ± 0.31% ^d
PIBRAC	5.33% ± 1.53% ^{bc}	1.73% ± 0.42% ^b
PROMITOR	4.00% ± 1.00% ^{bc}	1.67% ± 0.42% ^b
ROCAILLOU	5.33% ± 1.53% ^c	1.73% ± 0.46% ^b
RUBISKO	0.67% ± 0.58% ^a	0.13% ± 0.12% ^a
SACRAMENTO	0.00% ± 0.00% ^a	0.00% ± 0.00% ^a
SPHERE	5.67% ± 1.53% ^{cd}	2.21% ± 0.52% ^d
STK 3	2.33% ± 0.58% ^{ab}	0.87% ± 0.12% ^c
STK EXP	2.33% ± 1.53% ^{ab}	0.73% ± 0.50% ^d
TOSCA DOU	5.00% ± 1.00% ^c	1.07% ± 0.12% ^c
ULTIM	4.33% ± 0.58% ^{bc}	1.73% ± 0.31% ^b

Note: The letters (a, b, c, ..., g for Frequency(%); a, b, c, ..., k for DSI%) indicate homogeneous groups according to Tukey's HSD test. Mean values sharing the same letter are not significantly different ($p > 0.05$), while values with different letters are significantly different ($p < 0.05$). Letters for Frequency (%) and DSI (%) were determined separately.

Although frequency (%) provides information on the number of wheat kernels affected by black point, it does not reflect the intensity or degree of symptom severity for each affected kernel. To obtain a more comprehensive assessment of the disease impact, the Disease Severity Index (DSI%) was also calculated for each cultivar. The DSI values ranged from 0.00% in Sacramento to 5.53% in Datina, highlighting both highly tolerant and highly

susceptible cultivars. Cultivars such as Avenue (5.47%±1.10%), Asterion (4.80%±0.35%), and Boromir (4.60%±0.69%) showed high severity, while Complice (0.27%±0.31%), Rubisko (0.13%±0.12%), and Farneo (0.33%±0.12%) exhibited very low severity levels. Intermediate values were recorded for cultivars such as Adriatic, Anapurna, Apexus, Leonidus, and Extreme, indicating a moderate level of infection. Statistical analysis (ANOVA) revealed significant differences among cultivars for both frequency and DSI ($p < 0.001$). Tukey's HSD test allowed the grouping of cultivars into homogeneous subsets (letters a-g for frequency and a-k for DSI). For example, Datina, Avenue,

and Asterion formed the groups with the highest values (letters g or k), whereas Sacramento, Rubisko, and Complice formed the groups with the lowest values (letter a), highlighting significant differences between highly susceptible and highly tolerant cultivars.

The mycological analysis of wheat kernels showing black point symptoms revealed the presence of seven fungal genera associated with this disease. Among the fungal genera identified, *Alternaria* sp. was the most frequently detected, being present in the majority of the analysed varieties, confirming its important role in the development of black point symptoms in wheat (Table 5).

Table 5. Fungal species isolated from wheat kernels affected by black point

Winter wheat cultivar	<i>Alternaria</i> sp.	<i>Cladosporium</i> sp.	<i>Fusarium</i> sp.	<i>Epicoccum</i> sp.	<i>Curvularia</i> sp.	<i>Chaetomium</i> sp.	<i>Cochliobolus</i> sp.
ADRIATIC	+	-	+	+	-	-	+
AGENOR	+	-	+	+	-	-	+
ANAPURNA	+	-	+	+	-	-	+
APEXUS	+	-	+	+	-	-	+
APILCO	+	-	-	-	-	-	-
ARTIMUS	+	-	-	-	-	-	-
ASTERION	+	+	+	+	+	+	+
AVENUE	+	+	+	+	+	+	+
BASILIO	+	-	-	-	-	-	-
BOROMIR	+	+	+	+	+	-	+
CELEBRITY	+	-	+	+	-	-	+
CHEVIGNON	+	-	-	-	-	-	+
COMPLICE	+	-	-	-	-	-	-
DATINA	+	+	+	+	-	+	+
EXTREME	+	+	+	+	+	-	+
FALADO	+	-	-	-	-	-	+
FARNEO	+	-	-	-	-	-	-
GLIA	+	-	-	-	-	-	+
JUNIOR	+	-	-	-	-	-	-
LEONIDUS	+	-	+	+	-	-	+
MIX CEREALE	+	+	+	+	-	-	+
MUSIK	+	-	-	-	-	-	-
PASSION	+	-	-	-	-	-	-
PG 102	+	-	+	+	-	-	+
PIBRAC	+	-	+	+	-	-	+
PROMITOR	+	-	-	-	-	-	+
ROCAILLOU	+	-	+	+	-	-	+
RUBISKO	+	-	-	-	-	-	-
SACRAMENTO	-	-	-	-	-	-	-
SPHERE	+	+	+	+	-	-	+
STK 3	+	-	-	-	-	-	-
STK EXP	+	-	-	-	-	-	-
TOSCADOU	+	-	-	-	-	-	+
ULTIM	+	-	+	+	-	-	+

Note: Presence (+) and absence (-) indicate the occurrence of fungal genera associated with wheat kernels affected by black point.

Fusarium sp. was also isolated from several cultivars, particularly from those showing higher values of frequency and DSI, such as Asterion, Avenue, Boromir, and Datina. Fungal genera such as *Cladosporium* sp., *Epicoccum* sp., and *Cochliobolus* sp. were detected sporadically, being more frequent in varieties

with a higher level of infection. In contrast, *Curvularia* sp. and *Chaetomium* sp. were detected only in a few cases, suggesting a limited involvement in the pathogenic complex associated with black point under the conditions of this study. In some varieties with very low disease frequency, such as Sacramento,

Rubisko, and Complice, the presence of fungal genera was limited or absent.

CONCLUSIONS

The results obtained highlight significant differences among wheat cultivars regarding frequency and the severity of black point disease, indicating varying levels of susceptibility. Cultivars exhibiting high frequency and disease severity index proved to be more susceptible, while others showed greater tolerance under the experimental conditions. The fungal community analysis confirmed the complex nature of the disease, with several fungal genera associated with black point symptoms, among which *Alternaria* sp. was the most frequently detected. The types of studies should be conducted regularly, as certain growing seasons and climatic conditions can favor the development of fungal diseases, including the occurrence of black point in wheat.

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