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The Occurrence of Fungal Diseases in Maize in Organic Farming Versus an Integrated Management System

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Abstract: Organic farming is becoming increasingly popular because it leads to healthier products. Due to limitations on the use of chemical protection, however, plants may be more susceptible to pathogen attacks. Therefore, the aim of the study was to determine the occurrence of fungal diseases in maize grown in organic versus integrated systems. The field experiment was conducted during the years 2017–2019 in Puławy, Poland. Three maize varieties, Ambrosini, Smolitop and Ricardinio, were cultivated in two fields with a different crop production system. The incidence of fungal diseases, such as northern corn leaf blight, eyespot, common corn rust, corn smut and Fusarium ear rot, was assessed. Fungal isolates were collected from leaves and cobs with disease symptoms and identified microscopically and molecularly. In both cultivation systems, northern corn leaf blight and eyespot were the most common, while corn rust and fusariosis were seen more often in organic cultivation. *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium poae*, *Fusarium graminearum* and *Fusarium sporotrichioides* were the fungal species most frequently detected in the two systems. Additionally, *Fusarium verticillioides* was common in the organic system. Weather conditions, especially heavy rainfall and high air humidity, greatly influenced the incidence of such diseases.

Keywords: fungal diseases; maize cultivation; organic and integrated systems

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1. Introduction

Maize (*Zea mays* L.) is widely used in agriculture and the food industry all over the world, including Poland. Grain and maize silage are used mainly as feed in livestock production. In 2018, over 1.2 million hectares of maize were cultivated in Poland, of which 645,000 were allocated for grain production and 601,000 for silage [1]. Corn is also processed into a wide variety of food and industrial products.

The corn market is one of the most dynamically developing agricultural markets. This is due to the versatility of the use of corn. It is a spring crop, very tolerant to precropping, which in Polish conditions is gradually replacing winter crops after frost. In the south, southwest and west of Poland, varieties are grown for grain much later than in other regions of the country. To the contrary, in northern Poland, much earlier varieties with a lower yield potential are grown. When maize is used for silage, slightly later varieties are chosen, which are characterised by higher productivity in both grain yield and green matter per hectare [2].

Most of the plantations are exposed to a variety of infections, of which fungal ones are very frequent. The most common fungal foliar diseases in maize observed in Poland are: northern corn leaf blight caused by *Exserohilum turcicum* (syn. *Helminthosporium turcicum* (Pass.) K.J. Leonard & Suggs), eyespot caused by the fungus *Kabatiella zeae* Narita & Y. Hirats (syn. *Aureobasidium zeae* (Narita & Y. Hirats) Dingley) and common corn rust caused by an infection by *Puccinia sorghi* Schwein [3]. The lesions caused by these fungi may

become so numerous that the maize leaves are destroyed, leading to loss of yield due to lack of available carbohydrates in the grains [3].

Additionally, the occurrence of common smut caused by the fungus *Ustilago maydis* (DC.) Corda (syn. *Ustilago zeae* (Link) Unger) and head smut caused by *Sphacelotheca reiliana* Kühn was observed on maize fields in Poland [3]. All these infections, however, rarely result in financial loss.

Moreover, there are several fungal pathogens that cause ear and kernel rots in corn that may reduce yield and grain quality. A quite common disease in Poland is red ear rot, caused by *Fusarium graminearum* Schwabe. Another disease for cobs is Fusarium ear rot, also known as pink ear rot, caused by several *Fusarium* species, the most common of which include *Fusarium verticillioides* (Sacc.) Nirenberg (syn. *F. moniliforme* Sheldon) and *Fusarium proliferatum* (Matsushima) Nirenberg [4]. Among other fungal diseases for maize, Fusarium ear rot is crucial for the quantity and quality of grain products used in human and animal nutrition [5,6]. The *Fusarium* species produce metabolites called mycotoxins, which contaminate human food and animal feed as well as cause disorders of the digestive system and kidney and liver functions [7–10]. The most common mycotoxins produced by several toxigenic *Fusarium* species are deoxynivalenol, trichothecenes, zearalenone, fumonisins and moniliformin [11,12].

The severity of fungal infections in maize production is largely dependent on environmental conditions (weather, temperature, wind, rain) as well as the choice of cultural practice (no till/minimum till, corn-on-corn rotation, elimination of weed infestation) and farming system (organic or integrated). The susceptibility of varieties used for breeding also plays a significant role [3,12–14].

Maize can be grown in both integrated and organic systems [15,16]. The organic cultivation of plants reduces the costs of cultivation and is becoming increasingly popular among Polish farmers [17,18].

Organic farming is a farming system consisting of balanced crop and livestock production within the farm. It relies on the use of correct crop rotation and other natural methods to maintain or enhance biological activity and soil fertility, as well as on the selection of plant species, varieties and animal breeds, taking into account their natural resistance to disease. Organic farming not only plays an important role in producing pesticide-free food, but also enhances and maintains the biodiversity and natural values of agricultural production spaces. It also increases employment in rural areas through the amount of work needed to produce organic products [19]. In an integrated cultivation system, the use of plant protection products is allowed if the level of presence of the pest exceeds the harmfulness threshold. In contrast, the organic system excludes chemical plant protection products against pests and pathogens, and weeds are controlled mechanically.

Meanwhile, modern hybrid varieties of maize, adapted to more intensive growing conditions, may show less resistance to fungal diseases under less than optimal cultivation conditions. For this reason, it is necessary to assess maize varieties in terms of their suitability for organic cultivation.

The aim of the study was to assess fungal infection in maize grown in organic and integrated farming systems between the years 2017 and 2019. In addition, the species composition of fungi within the genus *Fusarium* and the incidence of *Alternaria alternata* (Fr.) Keissler in both systems of cultivation were analysed. Moreover, the study also includes a comparison of three maize varieties as regards their response to fungal pathogens in order to indicate which varieties can be recommended for cultivation in the organic system.

2. Materials and Methods

2.1. Field Conditions of Experiment

The experiment was conducted in the Osiny Experimental Station of the Institute of Soil Science and Plant Cultivation—State Research Institute in Puławy, Lubelskie Voivodeship, Poland (51°27′ N, 22°03′ E). One early maize variety, Ambrosini (KWS), and two mid-early varieties, Smolitop (HR Smolice) and Ricardinio (KWS) (Table 1), were culti-

vated in two fields using different farming systems: organic and integrated [20]. Each variety was grown in four replications that together covered an area of 1500 m^2. The plot size for each variety was 375 m^2. In organic farming, plants were fertilised with composted manure (40 t/ha) and weeds were mechanically restricted. Meanwhile, in integrated farming, both manure (30 t/ha) and mineral fertilisation: 150 kg/ha N (CH_4N_2O), 39.2 kg/ha P ($Ca(H_2PO_4)_2$) and 49.8 kg/ha K (KCl), were applied. Moreover, in integrated cropping, the seeds were treated before sowing with a fungicide containing the following active substances: fludioxonil–25 g/L and metalaxyl-M–9.7 g/L (Maxim XL 034.7 FS), and weeds were controlled with herbicides containing sulcotrione (Shado 300 SC–1 L/ha) and nicosulfuron (Innovate 250 EC–0.2 L/ha). The experiment extended over three years (2017–2019).

Table 1. Comparison of maize varieties used in the experiment.

Variety (Breeder)	FAO Number	Earliness of Varieties	Type of Hybrid	Kernel Type	Direction of Use	Disease Tolerance	
						Ustilago maydis	*Fusarium*
Ambrosini (KWS)	220/220	early	TC	flint-like	ensilage/grain	high	high
Ricardinio (KWS)	230/240	mid-early	SC	flint-like	ensilage/grain	high	very high
Smolitop (HR Smolice)	230	mid-early	TC	intermediate	ensilage/grain	high	high

TC—three-way cross; SC—single cross; KWS—KWS SAAT SE & Co. KGaA, international breeding company; HR Smolice—Hodowla Roślin Smolice, Polish breeding company.

2.2. Meteorological Data

During the growing season, in the period from April to the end of August, the mean air temperature, total rainfall and relative air humidity were recorded by an automatic weather station placed near the experimental site.

2.3. Observation of Disease Symptoms and Sampling for Research

The disease symptoms were observed in the second half of August on one hundred randomly selected plants from each farming system and each variety. The range of leaf and cob damage caused by fungal pathogens was determined using a 0–9 point scale, where 0 indicated no disease symptoms, 5 indicated that 10–15% of the leaf area or cobs (in the case of *Fusarium*) was affected by lesions, and 9 indicated that more than 50% of the leaf area or cob surface was covered by symptoms. The other degrees of the scale denoted intermediate symptoms. In order to monitor the fungal species present in plantations, leaf fragments and cobs with mould symptoms were collected from 49 corn plants (135 samples) from the organic system and 39 plants (103 samples) from the integrated system.

In the case of common smut, the percentage of plants infected by *U. maydis* was determined.

2.4. Decontamination of Plant Material and Obtaining Pure Cultures of Fungi

Leaf fragments and grains with symptoms of fungal disease were rinsed under tap water for 20 min and surface-disinfected in 70% ethanol (30 s). Afterwards, they were disinfected for 2–3 min in a 4% sodium hypochlorite solution (NaOCl; 7.4 g/mol) and rinsed three times in distilled water. Next, the plant material was dried on sterile filter paper, placed on half-concentrated potato dextrose agar (Potato Dextrose Agar Difco, Becton, Dickinson and Company, Sparks, MD, USA) in Petri dishes, supplemented with 2.5 mg/L chlortetracycline (tetracycline hydrochloride, Sigma-Aldrich, Darmstadt, Germany) and incubated at 25 °C in darkness. After 3–4 days, the mycelial hyphae that had grown from the leaf fragments or grains of maize were transferred to a fresh medium. These transfers were repeated several times until pure, homogeneous fungal isolates were obtained. The microscopic identification of isolates was performed using the Nikon Eclipse 80i microscope and taxonomic keys [21,22].

2.5. Fungal Species Identification

Representative fungal isolates morphologically different from each other in terms of growth rate, colour and mycelial structure were selected for DNA isolation. For this, small fragments of mycelium were scraped off the surface of the medium and were then collected into 2 mL sterile Eppendorf tubes with sterile homogenisation beads. Homogenisation was performed for 3 min at 30 Hz in a homogeniser (Tissue Lyser II, Qiagen Retsch GmbH, Haan, Germany). The fungal genomic DNA was isolated using a modified CTAB method [23]. An extraction buffer with the following composition was used: 3% v/v CTAB, 100 mM Tris-base, 20 mM EDTA and 1.4 M NaCl (pH = 8).

After extraction, the DNA concentration was measured using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). PCR was performed using various sets of primers obtained from the literature and designed for *A. alternata* and the genus *Fusarium*, including the following species: *F. oxysporum*, *F. poae*, *F. culmorum*, *F. graminearum*, *F. sporotrichioides*, *F. proliferatum*, *F. equiseti*, *F. avenaceum* and *F. solani*. The PCR reaction mixture in a volume of 20 µL contained: 1 × PCR buffer (75 mM Tris-HCl (pH = 8.8), 20 mM $(NH_4)_2SO_4$, 0.01% (*v/v*) Tween 20), 2.5 mM $MgCl_2$, 0.2 mM of each nucleotide, 0.5 U Taq polymerase (Thermo Fisher Scientific Baltic UAB, Vilnius, Lithuania), 0.5 mM of selected primers and 20 ng of genomic DNA. Fragments of DNA were amplified using C1000 and S1000 thermocyclers (Bio-Rad, Hercules, CA, USA). The primer sequences and temperature profiles used for the identification of genus *Fusarium* and individual species are given in Table 2. DNA extracted from fungal isolates obtained from the Pathogen Bank (at the Institute of Plant Protection—National Research Institute, Poznań, Poland) was used as positive and negative controls.

Table 2. Primers used and PCR conditions for determining fungus species.

Species Specificity	Primer	PCR Conditions	Product Size [bp]	Source
Fusarium spp.	ITS-Fu1, ITS-Fu2	95 °C 5 min; (94 °C 50 s, 56 °C 50 s, 72 °C 1 min) × 35; 72 °C 7 min	466	[24]
Fusarium avenaceum	JiAF, JiAR	95 °C 3 min; (94 °C 30 s, 58 °C 30 s, 72 °C 2 min) × 40; 72 °C 5 min	220	[25]
Fusarium culmorum	Fc01F, Fc01R	95 °C 5 min; (94 °C 20 s, 66 °C 1 min, 72 °C 45 s) × 5; (94 °C 20 s, 64 °C 1 min, 72 °C 45 s) × 5; (94 °C 20 s, 62 °C 1 min, 72 °C 45 s) × 25; 72 °C 5 min	570	[26]
Fusarium equiseti	Feq-F, Feq-R	95 °C 1 min 25 s; (95 °C 35 s, 66 °C 30 s, 72 °C 30 s) × 30, (95 °C 35 s, 54 °C 30 s, 72 °C 30 s) × 20; 72 °C 5 min	900	[27]
Fusarium graminearum	UBC85F, UBC85R	94 °C 2 min; (94 °C 1 min, 55 °C 1 min, 72 °C 2 min) × 40; 72 °C 5 min	332	[28]
Fusarium oxysporum	Fof1, Fof2	95 °C 5 min; (94 °C 50 s, 58 °C 50 s, 72 °C 1 min) × 35; 72 °C 7 min	340	[29]
Fusarium proliferatum	Pro1, Pro2	95 °C 5 min; (94 °C 50 s, 56 °C 50 s, 72 °C 1 min) × 35; 72 °C 7 min	585	[30]
Fusarium poae	Fp82F, Fp82R	94 °C 2 min; (94 °C 1 min, 55 °C 1 min, 72 °C 2 min) × 40; 72 °C 5 min	220	[31]
Fusarium sambucinum	FsF1, FsR1	94 °C 60 s; (94 °C 50 s, 58 °C 30 s, 72 °C 60 s) × 25; 72 °C 7 min	315	[29]
Neocosmospora solani (syn. *F. solani*)	TEF-Fs4F, TEF-Fs4R	95 °C 2 min; (95 °C 45 s, 58 °C 45 s, 72 °C 2 min) × 40; 72 °C 10 min	658	[24]
Fusarium sporotrichioides	FspIT2K, P28SL	95 °C 3 min; (95 °C 30 s, 68 °C 30 s, 72 °C 40 s) × 40; 72 °C 7 min	288	[32]
Fusarium verticillioides	Ver1, Ver2	95 °C 5 min; (94 °C 50 s, 56 °C 50 s, 72 °C 1 min) × 35; 72 °C 7 min	578	[30]
Alternaria alternata	AAF2, AAR3	95 °C 2 min; (95 °C 45 s, 58 °C 45 s, 72 °C 2 min) × 40; 72 °C 10 min	340	[33]

After amplification, the PCR products were separated by electrophoresis on a 2% agarose gel in 1 × TBE buffer with ethidium bromide. The presence and length of the PCR products were assessed through observation under UV light and comparison with a size standard (GeneRuler 100 bp DNA Ladder, Thermo Fisher Scientific Baltic UAB, Vilnius, Lithuania).

2.6. Statistical Analysis

Statistical analysis was run with Statistica 13.3. The percentage of plants infected with *U. maydis* was subjected to angular transformation [34]. Statistically significant differences were calculated using the ANOVA analysis of variance. The analysis of variance was performed at a significance level of $p \leq 0.05$.

In turn, as the degree of infestation by other pathogens was determined on an ordinal scale (0–9), the statistical analysis was run with the non-parametric Mann–Whitney U and Kruskal–Wallis tests showing significant differences in maize infestation between the two cultivation systems and three tested varieties, respectively. The value $p \leq 0.05$ was assumed for these tests.

3. Results

3.1. Weather Conditions

The mean air temperature, total rainfall and relative air humidity during the growing seasons from 2017 to 2019 are presented in Table 3. In the vegetative season from April to August 2017, total rainfall was 400 mm, in 2018 it was 277.5 mm and in 2019 it amounted to 280.9 mm. In 2017 and 2018, the highest rainfall occurred in July (119.6 mm and 122.5 mm, respectively), while the month with the highest rainfall in 2019 was August (86.7 mm). The mean air temperature during the growing season was the following: 15.5 °C in 2017, 18.1 °C in 2018 and 16.6 °C in 2019. The weather data show that the years in question were similar in terms of thermal conditions, whilst 2017 was different from 2018 and 2019 in terms of precipitation. It is worth noting that relative air humidity in 2018 was higher (70.6%) than in other years (53.8% in 2017 and 52.12% in 2019).

Table 3. Mean air temperature, total rainfall and relative air humidity during the growing seasons in the years 2017–2019 at the Experimental Station in Osiny.

Month	Ten-Day Period	Mean Air Temperature (°C)			Total Rainfall (mm)			Relative Air Humidity (%)			Mean Air Temperature (°C)	Total Rainfall (mm)	Relative Air Humidity (%)
		2017	2018	2019	2017	2018	2019	2017	2018	2019	1961–2017		
April	I	10.8	11.1	8.2	10.7	16.6	1.7	49.7	68	42.1	8.0	50	69
	II	5.5	14.7	7.1	9.6	7.7	3.2	54.4	70	39.9			
	III	6.2	15	13.4	51.4	5.5	30.6	64.7	64	58.1			
	monthly	7.5	13.6	9.6	71.7	29.8	35.5	56	67	47			
May	I	10	17.3	9.5	28.4	4.8	7.0	65.8	63	54.5	13.6	67	70
	II	14.4	15	13.7	0.0	35.4	65.9	48.6	77	77			
	III	16.9	19.1	15.5	39.1	19.2	13.2	55.5	68	69.5			
	monthly	13.8	17.2	12.9	67.5	59.4	86.1	57	69	67			
June	I	16.7	19.6	20.2	5.2	0.9	1.0	44.5	62	52.3	16.8	79	71
	II	17.5	20.1	22.9	16.2	3.9	23	54.7	69	53.1			
	III	20	16.8	22	12.2	33.3	14.7	53.7	74	48			
	monthly	20.0	18.8	21.7	33.6	38.1	38.7	51	68	51			
July	I	17.3	19.3	17.1	7.3	6.6	9.9	50.6	63	52.9	18.5	87	71
	II	18	19.5	17.1	101.4	64	4.3	57.4	86	44.8			
	III	20.4	23	21.7	10.9	51.9	19.7	52	74	43.4			
	monthly	18.6	20.6	18.6	119.6	122.5	33.9	53	74	47			
August	I	22.9	23.4	19.4	3.1	12.2	10.5	44.3	69	44.8	17.8	71	72
	II	19.8	21.2	19.8	100.1	7.1	72.6	55	75	52.9			
	III	16.5	17.8	21.3	4.4	8.4	3.6	56.2	77	48.5			
	monthly	19.7	20.8	20.2	107.6	27.7	86.7	52	74	49			

3.2. The Influence of the Cultivation System on the Incidence of Fungal Disease

The rate of fungal disease incidence in the two cultivation systems is presented in Table 4. The highest number of plants with symptoms of fungal disease was recorded in 2018 for both maize management systems. In turn, in 2017 and 2019, the lowest number of plants with disease symptoms was observed for the integrated system. Overall, northern corn leaf blight and eyespot dominated in both cultivation systems. The mean degree of infection by *E. turcicum* in 2018 was 5.63 in maize grown in the organic system and 6.59 in the integrated system. Infection with *K. zeae* in 2018 was more frequent in the organic system (where the mean degree of infestation was 5.38) than in the integrated one.

Table 4. Fungal disease occurrence in maize cultivated under organic and integrated systems of management in the years 2017–2019.

Disease	Cultivation System	Mean Degree of Plant Infestation		
		2017	2018	2019
Northern corn leaf blight	Organic	2.23 bA	5.63 bC	5.07 aB
	Integrated	2.69 aA	6.59 aC	3.84 bB
Eyespot	Organic	1.21 aA	5.38 aC	2.41 aB
	Integrated	1.36 aB	4.21 bC	0.2 bA
Common corn rust	Organic	0.52 aB	0.59 aC	0.03 aA
	Integrated	0.11 bAB	0.46 aA	0.01 aB
Fusarium ear rot	Organic	0.54 aA	0.87 bA	0.80 aA
	Integrated	0.30 bA	1.38 aC	0.44 bB

Different lowercase letters denote significant statistical differences between cultivation systems in the same year (according to the Mann–Whitney test), while different capital letters denote statistical differences among years (according to the Kruskal–Wallis test) at a probability level of $p \leq 0.05$.

The highest mean degree of corn cob infection by *Fusarium* species was observed in the integrated system in 2018 (1.38). The lowest number of plants infected by the *Fusarium* species was observed in 2017 among plants grown in the integrated system, where the mean degree of infestation was 0.30.

Common corn rust was shown to be a negligible problem in the tested fields. The severity of common corn rust was greater in the organic maize growing system, but the difference between management systems was shown to be statistically significant only in 2017. The greatest infestation was recorded in 2018 on the organic farm.

All three varieties under the organic system were more severely affected by eyespot than in integrated farming (Table 5). In addition, common corn rust was more prevalent in plants of the Ricardinio and Smolitop varieties cultivated according to organic farming. In contrast to the other varieties, the level of common rust infestation in the Ambrosini variety was low regardless of the cropping system. The rest of the fungal diseases affected the tested varieties at a similar level, and no statistical differences were reported (Tables 5 and 6).

3.3. Northern Corn Leaf Blight

The greatest severity of the disease was observed in 2018 in both organic and integrated farming systems (Table 4). In 2019, the organic standards applied in the cultivation of maize resulted in a higher degree of infection of plants by *E. turcicum* than was noted in the field under integrated cultivation (5.07 and 3.84, respectively). The mean degree of plant infestation was the lowest in 2017 in the organic field and amounted to only 2.23. In both 2017 and 2018, the Ambrosini variety cultivated according to organic methods reacted with the highest degree of infestation (Figure 1). This variety, however, was also shown to be susceptible to *E. turcicum* in the integrated management system. Meanwhile, Smolitop was the least affected, with the exception of the 2019 organic yields.

Table 5. The infestation of maize varieties depending on the cultivation system in the years 2017–2019.

Disease	Variety	Mean Degree of Plant Infestation in Each Cultivation System in 2017–2019	
		Organic	Integrated
Northern corn leaf blight	Ambrosini	5.39 a	5.57 a
	Ricardinio	4.47 a	4.09 a
	Smolitop	3.24 a	4.02 a
Eyespot	Ambrosini	3.02 a	2.16 b
	Ricardinio	2.71 a	1.85 b
	Smolitop	3.26 a	1.77 b
Common corn rust	Ambrosini	0.04 a	0.04 a
	Ricardinio	0.18 a	0.04 b
	Smolitop	0.92 a	0.49 b
Fusarium ear rot	Ambrosini	0.73 a	0.73 a
	Ricardinio	0.44 a	0.58 a
	Smolitop	1.05 a	0.8 a

Different letters in rows mean significant statistical differences according to the Mann–Whitney test at $p \leq 0.05$.

Table 6. The infestation of maize varieties with corn smut depending on the cultivation system.

Variety	Percentage of Infested Plants in 2017–2019 in Each Cultivation System	
	Organic	Integrated
Ambrosini	1.01	5.70
Ricardinio	3.68	4.33
Smolitop	5.67	8.00

No statistically significant differences were observed between systems according to the ANOVA analysis of variance at $p \leq 0.05$.

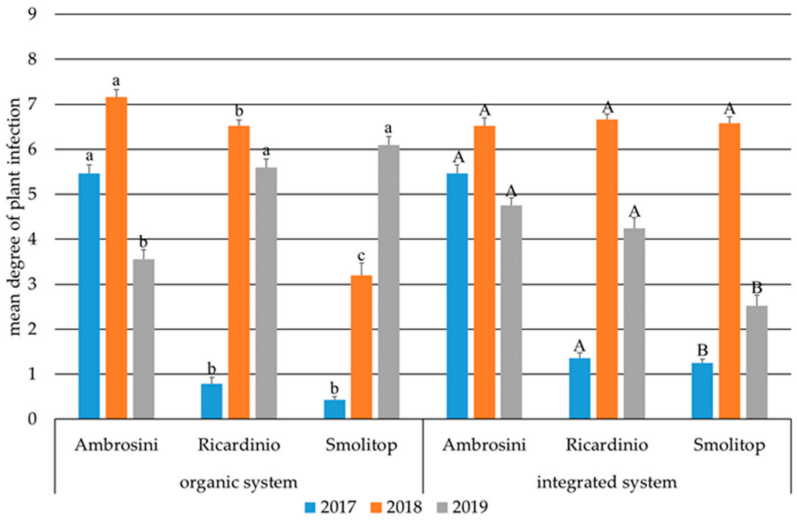


Figure 1. Plant infestation (mean ± SEM) with northern corn leaf blight of three maize varieties in the years 2017–2019 in two cultivation systems; different letters denote significant differences among varieties (Ambrosini, Ricardinio, Smolitop) in the same year for organic (lowercase letters) and integrated (capital letters) farming, according to the Kruskal–Wallis test at $p \leq 0.05$.

3.4. Eyespot

The pathogen causing this disease demonstrated the highest degree of infection in 2018 in both organic and integrated systems (5.38 and 4.21, respectively). In 2019, the mean degree of plant infestation in the organic system was also high, at 2.41 (Table 4). The maize varieties used in the experiment were susceptible to infection at an almost equal degree in both growing systems. The Ricardinio variety grown in the integrated system, however, was the least affected by the fungus *K. zeae* in 2019 (Figure 2).

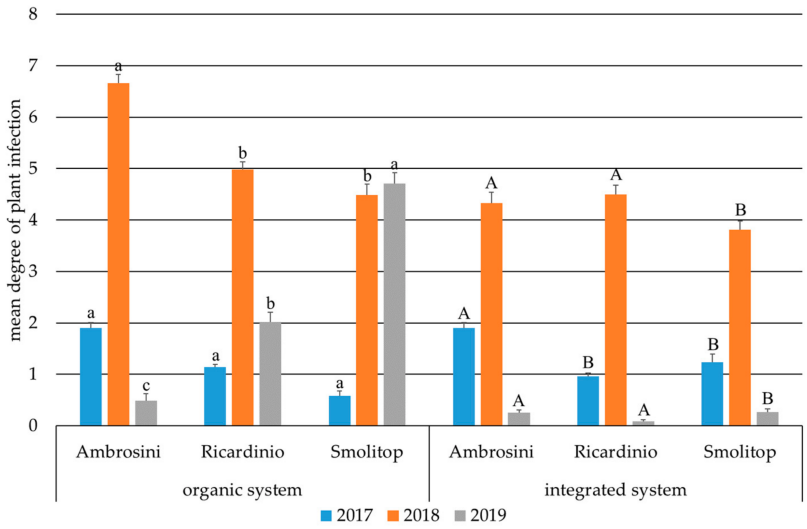


Figure 2. Plant infestation (mean ± SEM) with eyespot of three maize varieties in the years 2017–2019 in two cultivation systems; different letters denote significant differences among varieties (Ambrosini, Ricardinio, Smolitop) in the same year for organic (lowercase letters) and integrated (capital letters) farming, according to the Kruskal–Wallis test at $p \leq 0.05$.

3.5. Common Corn Rust

The appearance of *P. sorghi* on corn plantations in 2017–2019 was not extensive. The severity of infection observed was greater in organic farming than in integrated systems. In 2018, the mean degree of plant infection by *P. sorghi* amounted to 0.59 in organic farming, while in integrated cultivation, it was 0.46 (Table 4). The Smolitop corn variety proved to be the most susceptible to *P. sorghi* infection (Figure 3).

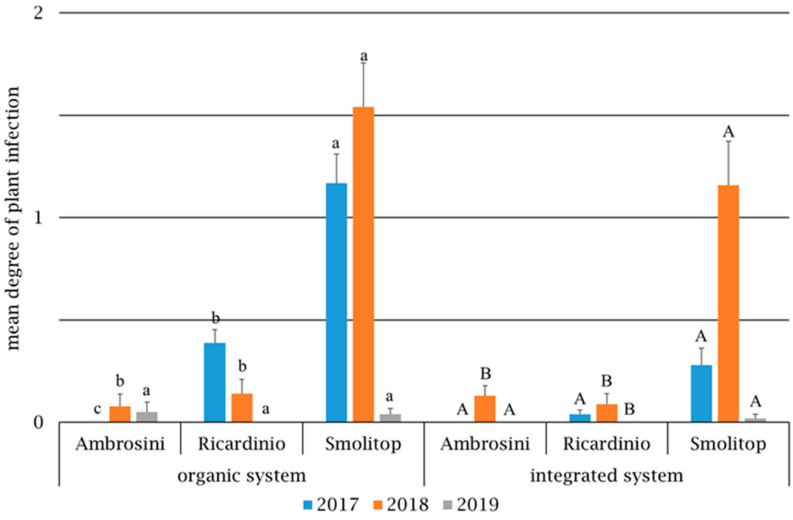


Figure 3. Plant infection (mean ± SEM) with corn rust of three maize varieties in the years 2017–2019 in two cultivation systems; different letters denote significant differences among varieties (Ambrosini, Ricardinio, Smolitop) in the same year for organic (lowercase letters) and integrated (capital letters) farming, according to the Kruskal–Wallis test at $p \leq 0.05$.

3.6. Fusarium Ear Rot and Red Ear Rot

Fusarium ear rot and red ear rot are diseases caused by fungi of the genus *Fusarium*. In the years 2017 and 2019, cob fusariosis was more prominent in the corn plantation managed by the organic system than in the integrated one (Table 4). In 2018, the highest mean degree of corn cob infection was observed in the integrated system (1.38). There was no infection

of maize cobs of the Ricardinio variety by any *Fusarium* species in the integrated system in 2019 (Figure 4).

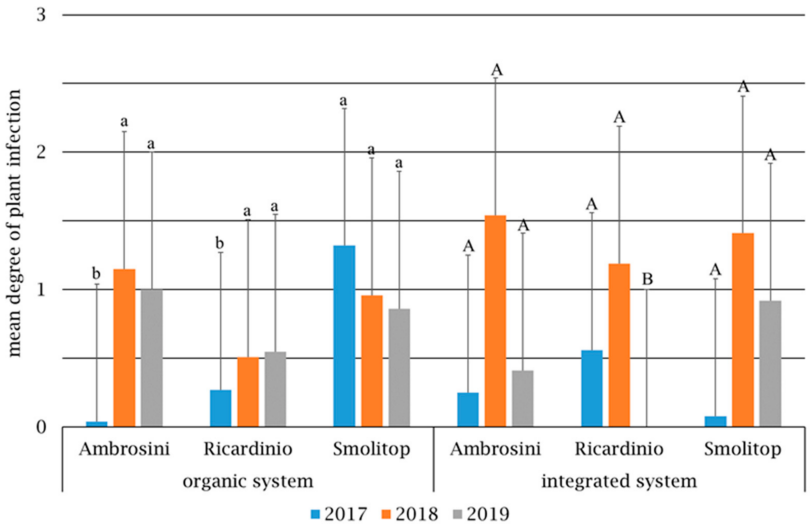


Figure 4. Infestation of maize cobs (mean ± SEM) by *Fusarium* species of three maize varieties in the years 2017–2019 in two cultivation systems; different letters mean significant differences among varieties (Ambrosini, Ricardinio, Smolitop) in the same year for organic (lowercase letters) and integrated (capital letters) farming, according to the Kruskal–Wallis test at $p \leq 0.05$.

3.7. Corn Smut

In both growing systems, *U. maydis* attacked individual plants. No statistically significant differences were found between cultivation methods or among years (Figure 5, Table 6). In the organic system, the percentage of plants with smut was the highest in 2018 (7.07%; Figure 5). In the organic system, Smolitop was the most affected by *U. maydis* among the tested maize cultivars. In 2019, however, corn smut was significantly more prevalent in the integrated system (14.28% of plants were infected), with Smolitop being the worst affected while the percentage of infected plants belonging to the varieties Ambrosini and Ricardinio was 6 and 8%, respectively.

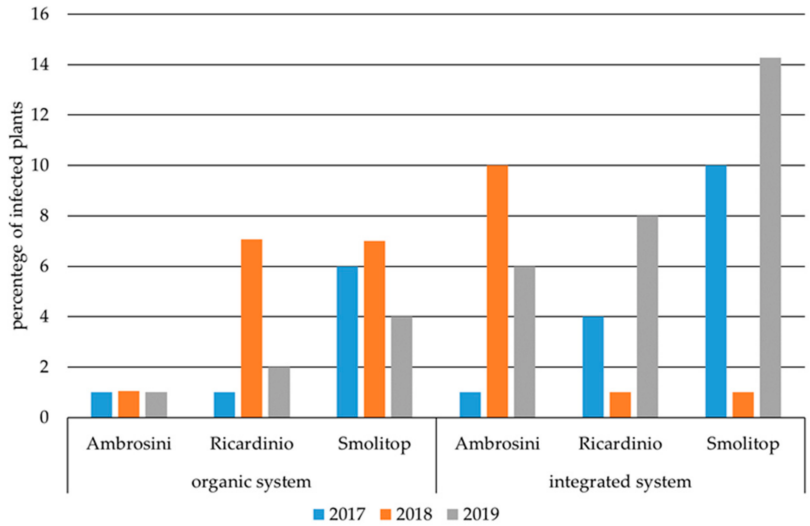


Figure 5. Percentage of plants with corn smut in three maize varieties (Ambrosini, Ricardinio, Smolitop) in the years 2017–2019 in two cultivation systems. There were no significant differences among varieties within cultivation systems, according to the ANOVA analysis of variance at $p \leq 0.05$.

3.8. Fungi Isolated from Infected Maize Plants

Maize plants were colonised by both pathogenic and saprotrophic fungi. A total of 172 fungal isolates were obtained in the organic cropping system (Table 7) and 147 isolates of fungi (Table 8) from plants cultivated in the integrated system between 2017 and 2019. In 2018, the highest number of fungal isolates were obtained from infected maize leaves and cobs in both farming systems (83 and 66 respectively). *A. alternate* and *Fusarium* spp. fungi were isolated from the tested samples. The predominant genus identified in organic maize was *A. alternata* (51.2% of isolates) followed by *Fusarium* (48.8%; Table 7). In contrast, the predominant genus isolated from the integrated system was *Fusarium* (64.7% of isolates), followed by *A. alternata* (35.4%). From maize cultivated in organic farming, we obtained fungi from the genus *Alternaria*, represented by the *A. alternata* (51.2% of isolates), and the genus *Fusarium*, represented by the following species: *F. oxysporum* (22.1%; Figures 6 and 7), *F. verticillioides* (2.9%), *F. graminearum* (2.3%), *F. poae* (2.3%), *F. sporotrichioides* (1.2%), *F. avenaceum* (0.6%) and other *Fusarium* spp. that have not been classified (17.4%).

Table 7. Fungi isolated from infected maize plants from 2017 to 2019 in the organic cropping system.

Species	Isolates Obtained in Organic Farming							
	2017		2018		2019		2017–2019	
	F	[%]	F	[%]	F	[%]	F	[%]
	n = 15		*n* = 54		*n* = 66		*n* = 135	
Alternaria alternata (Fr.) Keissler	11	47.83	38	45.8	39	59.1	88	51.2
Fusarium avenaceum (Fr.) Sacc.	0	0	0	0	1	1.51	1	0.6
Fusarium culmorum (Wm. G. Sm.) Sacc.	0	0	0	0	0	0	0	0
Fusarium equiseti (Corda) Sacc.	0	0	0	0	0	0	0	0
Fusarium fujikuroi Nirenberg	0	0	0	0	0	0	0	0
Fusarium graminearum Schwabe	2	8.7	1	1.2	1	1.51	4	2.3
Fusarium oxysporum Schltdl.	4	17.4	15	18.1	19	28.8	38	22.1
Fusarium poae (Peck) Wollenw.	0	0	4	4.82	0	0	4	2.3
Neocosmospora solani (Mart.) L. Lombard & Crous [syn. *F. solani* (Mart.) Sacc.]	0	0	0	0	0	0	0	0
Fusarium sambucinum Fuckel	0	0	0	0	0	0	0	0
Fusarium sporotrichioides Sherb.	2	8.7	0	0	0	0	2	1.2
Fusarium verticillioides (Sacc.) Nirenberg	0	0	5	6.02	0	0	5	2.9
Other *Fusarium* spp.	4	17.4	20	24.1	6	9.1	30	17.4
Total isolates obtained	23	–	83	–	66	–	172	100

F—frequency; *n*—number of collected plant samples.

Table 8. Fungi isolated from infected maize plants from 2017 to 2019 in the integrated cropping system.

Species	Isolates Obtained in Integrated Farming							
	2017		2018		2019		2017–2019	
	F	[%]	F	[%]	F	[%]	F	[%]
	n = 15		*n* = 42		*n* = 46		*n* = 103	
Alternaria alternata (Fr.) Keissler	4	11.43	14	21.21	34	74	52	35.4
Fusarium avenaceum (Fr.) Sacc.	0	0	2	3.03	0	0	2	1.4
Fusarium culmorum (Wm. G. Sm.) Sacc.	0	0	0	0	0	0	0	0
Fusarium equiseti (Corda) Sacc.	0	0	0	0	1	2.2	1	0.7
Fusarium fujikuroi Nirenberg	0	0	0	0	0	0	0	0
Fusarium graminearum Schwabe	0	0	8	12.12	1	2.2	9	6.1
Fusarium oxysporum Schltdl.	18	51.43	14	21.21	2	4.35	34	23.1
Fusarium poae (Peck) Wollenw.	3	8.6	11	16.7	2	4.35	16	10.9
Neocosmospora solani (Mart.) L. Lombard & Crous [syn. *F. solani* (Mart.) Sacc.]	0	0	0	0	1	2.2	1	0.7
Fusarium sambucinum Fuckel	0	0	0	0	0	0	0	0
Fusarium sporotrichioides Sherb.	4	11.43	0	0	0	0	4	2.7
Fusarium verticillioides (Sacc.) Nirenberg	0	0	2	3.03	0	0	2	1.4
Other *Fusarium* spp.	6	17.14	15	22.73	5	11	26	17.7
Total isolates obtained	35	–	66	–	46	–	147	100

F—frequency; *n*—number of collected plant samples.

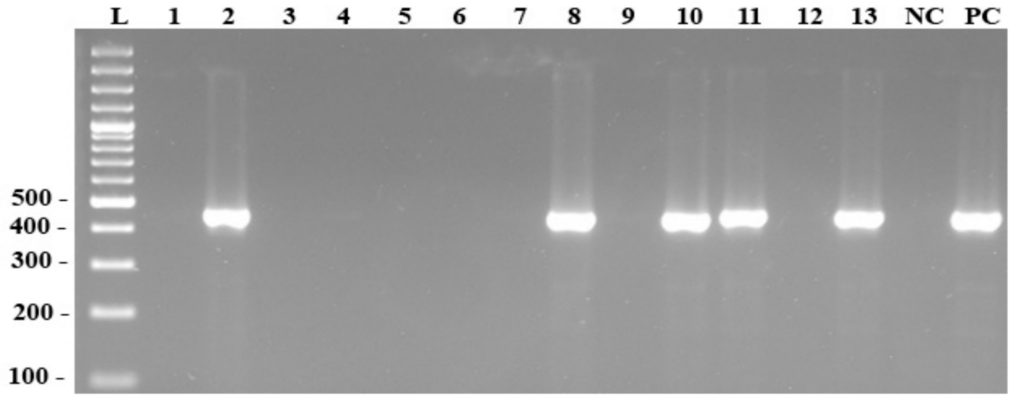


Figure 6. Electrophoretic separation of PCR products (466 bp) obtained with the use of *Fusarium* spp. specific primers ITS Fu1/Fu2; L—GeneRuler 100-bp DNA Ladder, 1–13—tested samples, NC—negative control, PC—positive control.

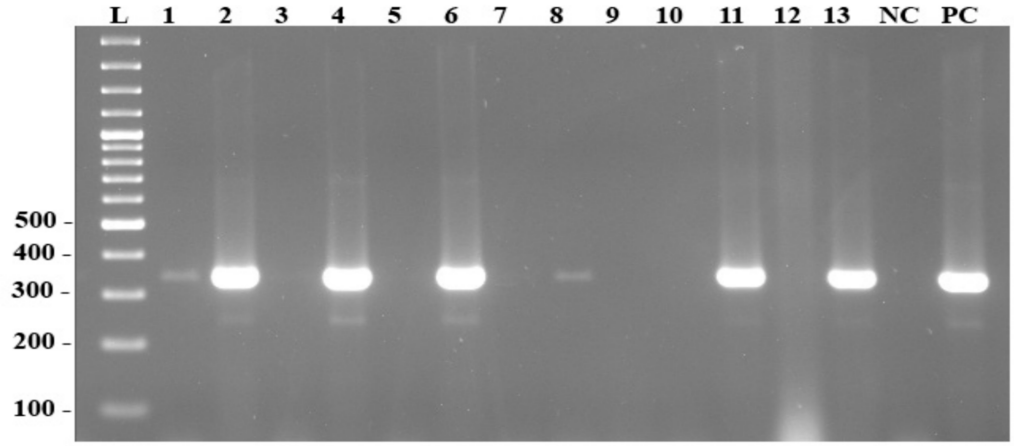


Figure 7. Electrophoretic separation of PCR products (340 bp) obtained with the use of Fof1/Fof2 primers specific for *F. oxysporum*; L—GeneRuler 100-bp DNA Ladder, 1–13—tested samples, NC—negative control, PC—positive control.

The dominant species, *A. alternata* (35.4% of isolates) and *F. oxysporum* (23.1%), *F. poae* (10.9%) and *F. graminearum* (6.1%), in more often colonised maize cultivated in integrated farms (Table 8).

4. Discussion

In the present study, the highest incidence of fungal diseases in maize in both cropping systems was observed in 2018, when heavy rainfall and high relative humidity were recorded, especially in the second ten-day period of July and in August. It is probable that the high air humidity and the difference in the amount of precipitation between July and August had an impact on the frequency and occurrence of fungal diseases in maize plantations. This could explain the presence of *E. turcicum* infections in both maize systems in 2018 because it thrives during periods of moderate (temperature between 20 and 28 °C), wet and humid weather [35]. In 2017, rainfalls were also plentiful but short in duration and limited to the second ten-day period of the month. Meanwhile, in July 2018, rainfalls were more regular, which resulted in high relative humidity persisting for a long time. Such weather conditions favoured the spread of fungal diseases. It is possible that the influence of humidity was so great that it nullified the importance of other factors. Simultaneously, in 2018, a high yield of seeds was recorded by Księżak [20], which means that fungal infestation did not reduce the yield. In our study, maize grown in 2019 in the organic

system had a higher rate of fungal disease infestation. This can be related to the high amount of rainfall in August, when the samples were collected, and is also confirmed by the Księżak study [20], where it is recorded that the 2019 maize yield was lower in organic farming, perhaps as a result of the fungal infestation. According to Księżak [20], the maize varieties studied (Ambrosini, Sylvestre, Smolitop and Ricardinio), which were grown in the integrated system in 2017–2019, yielded on average 12.1% more seeds than those grown in the organic system.

The greatest differences in northern corn leaf blight and eyespot infestation emerged in 2019, when the relatively dry July and August weather was not very conducive to the development of fungal infections. In this case, the average degree of plant infection was distinctly higher in the organic cultivation system. The year 2019 was also marked by a clear difference in yields, which were lower in the case of plantations managed according to organic cultivation rules [20].

In 2017–2019, *F. oxysporum*, *F. verticillioides, F. graminearum* and *F. poae* were the most frequent *Fusarium* species isolated from maize cultivated in both organic and integrated systems. There are many reports on climate change determining *Fusarium* species population variability and *F. verticillioides* distribution in Central European countries [8,36,37]. Numerous studies confirm that moderate temperatures and a high level of moisture increase the infection rates of fungal diseases, especially the *Fusarium* species [8,14,38–40]. Red ear rot is caused by infections with *F. graminearum*, *F. culmorum* and *F. avenaceum* during periods of moderate temperatures and high humidity [40,41], while pink ear rot, induced by pathogens such as *F. verticillioides*, *F. proliferatum* and *F. temperatum*, has often been reported in southern regions of Europe where it was dry and warm [42].

Fungi of the genus *Fusarium* do not exist in isolation but compete with other microorganisms for niches in soil, plant debris and host plants. Their interactions and the balance between microbial communities are influenced by the prevailing environmental conditions. Temperature, water availability (a_w), aeration and light are key climatic factors influencing the production of *Fusarium* inoculum. Marín et al. [43,44] showed that temperature and a_w significantly influence the growth and interaction between *F. moniliforme* and *F. proliferatum* and among *F. graminearum*, *F. subglutinans*, *F. proliferatum*, *Aspergillus*, *Penicillium* and *Trichoderma* species. According to Fadiji et al. [45], organic farming has a direct impact on the diversity, functions and abundance of soil microbial communities and, thus, may be associated with improved yield, growth, enhanced plant resistance to abiotic and biotic stresses as well as improved soil health.

The predominant genus identified in organic maize was *Alternaria*, but fungi from the *Fusarium* genus were most commonly isolated from the integrated system. There are many reports in the literature comparing organic and conventional maize with wheat cultivation systems for the incidence of mycotoxin-producing fungi [46–48]. Ariño et al. [46] produced similar results to ours by obtaining more isolates of *Alternaria* fungi in organic cultivation. They reported that organic maize showed a higher total fungal incidence but lower contamination of kernels compared to conventional maize. It is possible that the higher number of *Alternaria* isolates in the organic system is related to the higher incidence of weeds, since herbicides as well as fungicides are prohibited in this type of growing. In our study, the sampling date in August coincided with the optimal period for *Alternaria* spores, which is July–August. Furthermore, *Alternaria* spores are more prevalent in manure-fertilised soils. Similarly, Lazzaro et al. [48] observed that contamination by *Fusarium* spp. was higher in conventional than in organic wheat. Bernhoft et al. [47] also found a significantly higher incidence of *Fusarium* spp. occurring in conventional Norwegian cereals, including wheat, compared to organic crops. The increased frequency of the presence of *Fusarium* fungi in conventional cereal crops, including wheat, may be due to the use of ineffective pesticides against *Fusarium* and mineral fertilisation. The intensive application of fungicides on cereals has selected for resistance in many cereal pathogens. Talas and McDonald [49] conducted studies on the fungicide resistance of *F. graminearum* isolates in a field population. Additionally, in the study by Yin et al., tebuconazole resistance

was found in 1 isolate among 41 Chinese *F. graminearum* isolates collected from 14 different locations [50]. The main factors differentiating between conventional and organic farming are the use of pesticides to protect plants against pathogens and mineral fertilisation. Many studies are, thus, available on this subject [51–53]. Research conducted by Henriksen and Elen [52] found that large amounts of nitrogen fertiliser applied to wheat resulted in increased crop densities and a change in the canopy microclimate, which caused high moisture content and, therefore, more favourable conditions for the emergence of *Fusarium* infection. In addition, Szulc et al. [54] found that the highest infestation of corn plants by *Fusarium* fungi occurred when broadcast seeding of NP fertiliser was applied. In contrast, the lowest plant infestation was observed when in-row sowing of NP fertiliser was applied at a depth of 15 cm.

Comparing the results of the above studies, however, it should be stated that, in general, organic maize cultivation is more exposed to attacks from fungal pathogens, primarily because the use of chemical plant protection products, in particular insecticides, is prohibited in this system. Mazzoni et al. [55] demonstrated the effectiveness of insecticides in reducing the infection of maize cobs by larvae. In addition, according to numerous reports, foraging by the European corn borer (*Ostrinia nubilalis*) and western corn borer (*Diabrotica virgifera*) in corn-growing areas promotes the spread of fungal diseases and, especially, the development of cob fusariosis [56–59]. In Szulc's study, it was shown that as damage to maize plants due to *O. nubilalis* increased, infestation by fungi of the genus *Fusarium* spp. also increased [60]. Mechanical damage caused by insect feeding allows the fungal pathogens to penetrate more easily into plant tissues. In the present study, we also observed a relationship between the occurrence of European corn borer larvae and the greater intensity of cob infestation by fungi of the genus *Fusarium* in organic farming. Further analyses, however, were not conducted to confirm this fact.

Out of the three studied varieties, Ambrosini seems to be the best suited to organic cultivation. Only in the case of eyespot was this variety infected to a significantly greater degree under organic conditions than in the integrated system. In terms of the rest of the diseases, Ambrosini performed comparably in both cultivation systems. The Ricardinio variety turned out to be relatively resistant to fusariosis and rust, in contrast to Smolitop. The latter variety was also characterised by low seed yield, as well as seed weight and number of grains per cob [20]. These differences, however, are rather a result of the genetic diversity and yielding abilities among the varieties. Nevertheless, the low yield of Smolitop may also be the result of its greater susceptibility to fungal diseases.

The use of appropriate agricultural practices in the cultivation of maize in either an organic or integrated manner contributes to the occurrence or reduction of fungal infections. In an organic system, tilling the soil between crops is necessary as a weed control technique because the use of herbicides is prohibited. It is clear that the most important inoculum sources for *Fusarium* spp. are plant debris and, especially, maize stalks. This is confirmed by many studies [38,61,62]. Vasileiadis et al. [62] showed that, after conventional ploughing, the incidence of *F. graminearum*, *F. temperatum* and *F. culmorum* was reduced, while the incidence of *F. verticillioides* and *F. equiseti* was enhanced by tillage. Steinkellner et al. [63], however, reported that the composition of *Fusarium* species is different depending on the soil layer and survival structures of the species. According to Nyvall and Kommedahl [64], *F. verticillioides* has the ability to survive in maize stalks at a depth of 30 cm due to higher soil moisture and poor decomposition of plant tissue. In our study, the incidence of *F. vertocillioides* was noted in 2018 when the abundance of rainfall and air humidity was relatively high. Therefore, conditions favourable for the survival of the pathogen in harvest residues were present.

Conversely, there are relatively few studies on the occurrence of fungal diseases in maize in both organic and integrated systems. In integrated pest management, mineral fertilisation and chemical plant protection agents are used if the threshold of financial losses by pathogens or pests is exceeded. Such a practice limits the use of chemical plant protection products to the necessary minimum, thus reducing pressure on the natural environment

and protecting the biodiversity of the agricultural environment. In our study on integrated maize cultivation, fungicides and insecticides were not used because the incidence of pathogenic fungi and pests did not exceed the thresholds of harmfulness. Therefore, our study did not include one of the factors that differentiate organic and integrated systems.

Taking the above into account, it is difficult to relate the results of our research to the existing literature comparing organic systems of cultivation with conventional ones. Therefore, there is a need to continue research on the incidence of fungal diseases on maize plants grown under organic and integrated systems in conditions prevalent in Poland. Moreover, the study should be extended to include other observations, for example the presence of mycotoxins in maize seed from plantations managed according to both farming methods.

5. Conclusions

A large amount of rainfall and high air humidity, especially in the second ten-day period of July and in August 2018, created favourable conditions for the development of fungal diseases in maize. This resulted in the highest severity of fungal disease on the evaluated corn varieties, regardless of the cropping system used. The clearest differences in infestation with fungal disease in the two cultivation systems emerged in 2019, which was characterised by a relatively dry summer. At that time, corn cultivated on the organic farm showed the highest susceptibility to disease. This was probably due to the greater weed infestation in the organic system and the use of a higher rate of composted manure. High plant density caused by weed infestation created a specific microclimate and thus promoted the incidence of fungal diseases. The obtained results have shown that, in terms of the occurrence of fungal diseases in maize, the organic system does not do remarkably worse than the integrated one. Only in years with weather conditions conducive to disease the integrated system outperformed organic farming. On this basis, it can be concluded that in dry years (with an average amount of precipitation lower than the multi-year data), in order to protect plants from fungal diseases, it is advisable to apply agricultural practices characteristic of the integrated farming system.

Moreover, earliness of a variety can play a role in plant disease susceptibility. The early varieties, such as Ambrosini, better tolerate cultivation in the organic system, while later-maturing ones, such as mid-early Smolitop, seem to be less suited for these conditions.

The study showed that the most common fungal diseases were northern corn leaf blight and eyespot, which caused high infestation in both organic and integrated cropping systems, while corn rust and fusariosis were more common in the organic system.

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