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The occurrence of *Fusarium* spp. in green *Asparagus officinalis* L. spears

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Abstract: Due to changes in the climate and the existence of a wide range of *Asparagus officinalis* L. cultivars, it is necessary to identify their suitability for cultivation in Poland and investigate their susceptibility to infection by the most common pathogens. The identification of the species composition of fungi of the *Fusarium* genus found in green spears of edible *A. officinalis* cultivars and the factors contributing to their occurrence will enable the effective protection of these crops. The study was conducted on seven *A. officinalis* cultivars bred in different countries, which were dioecious cultivars with male and female specimens: 'Ariane' (Germany), 'Cipres' (France), 'Eposs' (Germany), as well as cultivars with male specimens only: 'Andreas' (France), 'Gynlim', 'Grolim' (Netherlands), and 'Hannibal' (Germany). The analysis of the composition of the fungi isolated from the green *A. officinalis* spears showed that most of the isolates belonged to the *Fusarium* genus (*F. culmorum* Wm.G. Sm., *F. equiseti* (Corda) Sacc., *F. oxysporum* Schltdl., *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, *F. solani* (Mart.) Sacc., and *F. fujikuroi* Nirenberg). Other fungal species (*Alternaria*, *Botrytis*, *Cladosporium*, *Penicillium*, and *Stemphylium*) were rarely isolated. The majority of the *Fusarium* genus isolates came from the spears of the 'Ariane' and 'Eposs' cultivars showing disease symptoms and from the spears of the 'Grolim' cultivar without showing disease symptoms. The fungi of the *Fusarium* genus colonised both the spears with and without disease symptoms, but there were always more isolates on the ones with disease symptoms. Fungi of the *Fusarium* genus occurred more often in the epidermis than in the parenchyma. *F. oxysporum* was the dominant fungus in the *A. officinalis* spears under analysis. The number of fungi isolates of the *Fusarium* genus collected from the green *A. officinalis* spears tended to increase at the consecutive harvest dates, which means that the spears harvested at the latest date (late June) were the most heavily colonised by fungi. All of the fungi isolates of the *Fusarium* genus collected from the spears exhibited pathogenicity against *A. officinalis* plants.

Keywords: asparagus; different cultivars; epidermis; fungi species; green spear; parenchyma

Various *Asparagus officinalis* cultivars are grown in Poland. They are selected according to their suitability to specific cultivation conditions and methods, the functional characteristics of a particular cultivar, such as being completely male or dioecious, qualitative characteristics, and resistance to diseases. The German (dioecious cultivars: 'Eposs', 'Huchels Alpha', 'Schwetzinger Meisterschuss', 'Rambo' and totally male cultivars: 'Vulkan', 'Ravel', 'Ramada', 'Ramos') and Dutch cultivars (male ones: 'Franklim', 'Gynlim',

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'Backlim', 'Grolim', 'Avalim', 'Herkolim') are the most common ones grown in Poland. The French cultivars, such as the dioecious 'Cipres' and the male 'Andreas', are less frequently recommended (Knaflewski 2005).

Numerous scientific centres all over the world conduct research on various problems concerning *A. officinalis* cultivation due to the increasing popularity of this vegetable in various countries on all continents where the climate is favourable to grow it (Knaflewski, Sadowski 1990; Krug 1999; Knaflewski 2005; Andrzejak, Werner 2006; Kalomira 2007; Weber et al. 2007; Nothandel et al. 2013; Elmer 2015; Andrzejak, Janowska 2021). Apart from the problems of the cultivation conditions, such as the soil and nutritional requirements, the optimal harvest times, and the selection of high-yield cultivars, researchers frequently investigate important plant health issues. There have been several publications describing diseases of *A. officinalis* caused by viruses – asparagus virus 1 (AV1), asparagus virus 2 (AV2), cucumber mosaic virus (Elmer 1996; Fiedorow 1996; Jaspers 1996) and *Phytophthora megasperma* var. *sojae* (Fallon, Fraser 1991). However, most diseases damaging *A. officinalis* shoots and cladodes are caused by fungal pathogens (Hsu, San 1969; Sadowski 1990; Blok, Bollen 1995; Elmer 1996; Sonoda et al. 1997). The authors of studies usually focus on fungi of the *Fusarium* genus, which are most often isolated from damaged crowns, spears and shoots on asparagus plantations in all regions of the world, where they cause severe losses (Grogan, Kimble 1959; Caron et al. 1985; Fantino 1990; Blok, Bollen 1996; Guerrero et al. 1999; Borrego-Benjumea et al. 2014; Kathe et al. 2019, Brizuela et al. 2020; Witaszak et al. 2020).

The aim of this study was to determine the identification of the species composition of fungi of the *Fusarium* genus found in green spears of edible *A. officinalis* cultivars.

MATERIALS AND METHODS

Research location. An *A. officinalis* L. plantation at the Experimental Station of the Department of the Faculty of Horticulture and Landscape Architecture (now the Faculty of Agriculture, Horticulture and Bioengineering), Poznań University of Life Sciences (52°24'14"N 16°55'25"E) was observed for the occurrence of pathogens in three consecutive years.

Plants and cultivation methods. The study was conducted on seven *A. officinalis* cultivars bred in different countries. These were the dioecious cultivars with male and female specimens: 'Ariane' (Germany), 'Cipres' (France), 'Eposs' (Germany), as well as cultivars with male specimens only: 'Andreas' (France), 'Gynlim', 'Grolim' (Netherlands), and 'Hannibal' (Germany). All of the cultivars grown on the plantation had green spears. The plants were grown on lessivé soil, valuation class IV, formed from loamy sand deposited on light clay. The plantation meteorological conditions are shown in Figure 1.

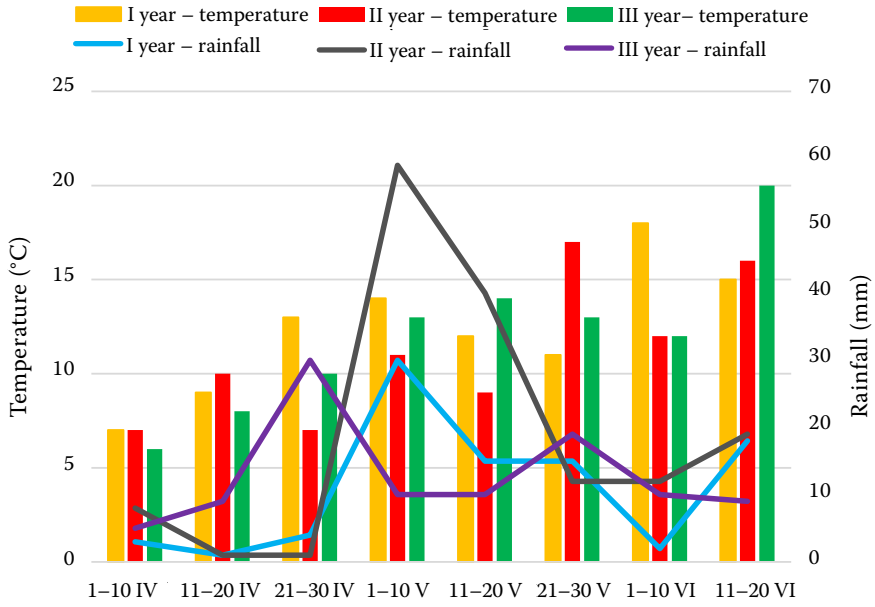

Figure 1. Meteorological conditions in the individual years of the study

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Sample preparation. Green spears of the *A. officinalis* cultivars were collected for tests in the fourth, fifth, and sixth year of cultivation. In the consecutive years, ten spears (repetitions) with visible disease symptoms at the base (brown irregularly-shaped stains or rusty spots) and ten disease-free spears (repetitions) were randomly selected and collected three times from each experiental field during the harvest time (on 2 and 26 May and on 20 June) (Figure 2). The samples were isolated from the spear bases, which were about 5 cm below the surface of the ground. On arrival from the plantation, the spears were thoroughly washed with tap water, placed on tissue paper and dried. Next, the spears were visually inspected in order to select fragments for isolation. The cut spear bases were disinfected with 1% sodium hypochlorite (NaOCl) for two minutes and then rinsed twice with sterile distilled water. After the disinfection, they were dried on sterile tissue paper again.

Five fragments with a diameter of 2 mm were cut from the epidermis of each spear as well as the parenchyma located directly below the epidermis. Next, the fragments collected from each spear were placed in Petri dishes on PDA (Merck) medium. When the agar was cooled down to about 45 °C, before it solidified, 16 ml of 0.05% streptomycin was added into each of the 90-mm Petri dishes (Weber, Knaflewski 2004). After solidification, the inoculum (five epidermis and five parenchyma fragments) was taken out and the dishes were placed in a thermostat at 20 °C for 14 days. The fungal cultures which developed at that time were inoculated on the obliquely solidified PDA agar in test tubes. The reinoculation resulted in cultures of the same age, which were segregated into similar groups, characterised macroscopically and microscopically, and identified according to mycological keys (Kwaśna et al. 1991; Leslie, Summerel 2006). The size, colour, and growth rate of the culture for 4–14 days on the solid substrate were observed on the PDA medium. The cultures were kept at 25 °C during the day and 20 °C at night on a 12-hour cycle. The shape, size of the conidia, conidiogenesis, type of phialide, and presence of microconidia and chlamydospores were observed on a Saltwater Nutrient Agar (SNA) medium. The taxonomic position of Fusarium spp. was determined according to the methodology described by Kwaśna et al. (1991) and Leslie and Summerell (2006). A group of isolates were selected for further investigation from the resulting cultures of the different species.

Experiment I – The occurrence of fungi of the *Fusarium* genus in the green spears of *A. officinalis*. The occurrence of fungi of the *Fusarium* genus in the green spears was compared according to the year of observation (I, II, III), the cultivar, the presence or absence of disease symptoms, the fragment of the tissue under analysis (epidermis, parenchyma) and the spear harvesting date (1., 2., 3.).

Experiment II – The pathogenicity of selected isolates of the *Fusarium* genus. The pathogenicity of the selected isolates of the *Fusarium* genus was assessed in experiments conducted in a growth chamber. The pathogenicity of the isolates collected from the green spears with and without disease symptoms was assessed. The group of isolates of various species

Figure 2. Green *Asparagus officinalis* spears
(A) – with disease symptoms; (B) – without symptoms

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of the *Fusarium* genus included items collected from the epidermis and the spear parenchyma. The list of the selected isolates is given in Table 1.

The pathogenicity of the selected isolates was assessed under controlled conditions. The experiment was conducted twice.

The research was conducted on the plants which developed from seeds of *A. officinalis* 'Andreas'. Before the seeds were placed on sprouters, they were disinfected in flasks containing acetone with 5% Benlate 50 WP. Next, the entire contents were placed on a shaker for 24 hours. Then, the disinfecting suspension was drained and the seeds were rinsed for another 24 hours in sterile distilled water. When the seeds germinated, they were transferred into 90-mm pots filled with a mixture of peat substrate and cold-frame soil (1 : 1 v/v).

A fungal inoculum was prepared on a 90-mm PDA medium disc and placed in the substrate following the method described by Mańka (1989). The variant with the lower inoculum was selected. When the *A. officinalis* seeds germinated, they were placed on the inoculum and covered with the substrate.

Experiment II conditions. The pots with the plants were placed in a growth chamber at a temperature of 24 °C during the illumination period and at 20 °C during the dark period. The plants were cyclically illuminated for 12 hours throughout the entire period. The observations were terminated after 16 weeks. During the experiment, the plants were regularly inspected every three days. Isolates were collected from the dying plants on the PDA medium. On the last day of cultivation, isolates were collected from all the other plants. The plants were cleaned from any remnants of the substrate. Next, the parts of the shoot with branches were cut off, whereas the remaining part of the shoot and the roots were disinfected. Then, they were dried and ten fragments of the shoot and root were cut out from each plant and placed on the solidified PDA medium in the Petri dishes. The dishes were incubated in a thermostat cabinet at 23 °C for 14 days. During this time, the emerging fungi cultures were split off and their species were identified on the basis of macro- and microscopic traits.

There were 17 growth chamber experiment treatments, each with 10 replications:

1. control variant A – absolute control (substrate without inoculum);
2. control variant B – methodical control (substrate with a disc of solidified PDA medium);

3. to 4. – substrate with an inoculum containing various *F. culmorum* isolates;

Table 1. The origin of the isolates of various species of the *Fusarium* genus assessed in the experiments conducted in the growth chamber

Species	Isolate symbol	Symptoms			
		yes		no	
		epidermis	parenchyma	epidermis	parenchyma
F. culmorum	F.c./Mgr	+			
	F.c./Mgr54			+	
F. equiseti	F.e./Mar2	+			
	F.e./Mgr9			+	
F. oxysporum	F.o./Mar7	+			
	F.o./Mg518		+		
	F.o./Mg982				+
	F.o./Mgr			+	
F. proliferatum	F.p./Mar3		+		
	F.p./Mgr1	+			
	F.p./Mgr3			+	
F. solani	F.s./Me01	+			
	F.s./Me02			+	
F. fujikuroi	F.v./Mgr51	+			
	F.v./Mgr58			+	

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5. to 6. – substrate with an inoculum containing various *F. equiseti* isolates;
7. to 10. – substrate with an inoculum containing various *F. oxysporum* isolates;
11. to 13. – substrate with an inoculum containing various *F. proliferatum* isolates;
14. to 15. – substrate with an inoculum containing various *F. solani* isolates;
16. to 17. – substrate with an inoculum containing various *F. fujikuroi* isolates.

Statistical analysis for experiment I. A three-way analysis of variance was applied to assess the occurrence of the fungi of the *Fusarium* genus in the green spears of the *A. officinalis* cultivars. There were separate calculations for both groups under study (the spears with and without disease symptoms). The following factors were analysed:
– the year of observation (I, II, III);
– the fungal species (*F. culmorum* Wm.G. Sm., *F. equiseti* (Corda) Sacc., *F. oxysporum* Schltdl., *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, *F. solani* (Mart.) Sacc., and *F. fujikuroi* Nirenberg);
– the cultivar ('Andreas', 'Ariane', 'Cipres', 'Eposs', 'Grolim', 'Gynlim', 'Hannibal').

The spears were subjected to a three-way analysis of variance to assess the occurrence of different species of the *Fusarium* genus in the epidermis and parenchyma of the individual cultivars. The results from all the study years were calculated separately for both groups (with and without disease symptoms). The following factors were analysed:
– the spear fragment (epidermis, parenchyma);
– the fungal species (*F. culmorum* Wm.G. Sm., *F. equiseti* (Corda) Sacc., *F. oxysporum* Schltdl., *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, *F. solani* (Mart.) Sacc., and *F. fujikuroi* Nirenberg);
– the cultivar ('Andreas', 'Ariane', 'Cipres', 'Eposs', 'Grolim', 'Gynlim', 'Hannibal').

The occurrence of the fungi of the *Fusarium* genus in the green *A. officinalis* spears of the different cultivars, depending on the harvest date, was assessed with a three-way analysis of variance. The results were calculated separately for both groups (with and without disease symptoms) and the consecutive years of the study. The following factors were analysed:
– the harvest date (1., 2., 3.);
– the fungal species (*F. culmorum* Wm.G. Sm., *F. equiseti* (Corda) Sacc., *F. oxysporum* Schltdl., *F. proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg, *F. solani* (Mart.) Sacc., and *F. fujikuroi* Nirenberg);
– the cultivar ('Andreas', 'Ariane', 'Cipres', 'Eposs', 'Grolim', 'Gynlim', 'Hannibal').

Additionally, a one-way analysis of variance was applied to determine the occurrence of the fungi of the *Fusarium* genus in the *A. officinalis* spears depending on the cultivar ('Andreas', 'Ariane', 'Cipres', 'Eposs', 'Grolim', 'Gynlim', 'Hannibal'). There were separate calculations for both groups under study (the spears with and without disease symptoms).

Statistical analysis for experiment II. The results of the two pot experiments were subjected to a one-way analysis of variance to assess the pathogenicity of the isolates of the *Fusarium* genus.

For experiment I and II, the software Statistica was used for the statistical calculations. The mean values were grouped with Duncan's test at a significance level of $\alpha = 0.05$.

RESULTS

The occurrence of fungi in the green *Asparagus officinalis* spears of different cultivars. During the three years of the study, six fungal genera were identified among the species. Most of the species belonged to the *Fusarium* genus. The others belonged to the following genera: *Alternaria, Botrytis, Cladosporium, Penicillium,* and *Stemphylium* (Figure 3).

The isolated species of the *Fusarium* genus and their counts. Table 2 lists the mean results for the three years of the study. Six fungal species of the *Fusarium* genus were isolated from the spears: *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. fujikuroi*. There were no statistically significant differences in the colonisation of the spears by the fungi of the *Fusarium* genus in the individual years of the study.

F. oxysporum and *F. proliferatum* were the most common species isolated from the group of spears with disease symptoms. There were statistically significant differences in the colonisation of the *A. officinalis* spears by these species in the 'Eposs' and 'Ariane' cultivars. *F. oxysporum* was also the most abundant species isolated from the spears without disease symptoms. The other species of the *Fusarium* genus were isolated sporadically. In this group of spears, the 'Gynlim' and 'Eposs' cultivars differed significantly in the fungal colonisation (Table 2).

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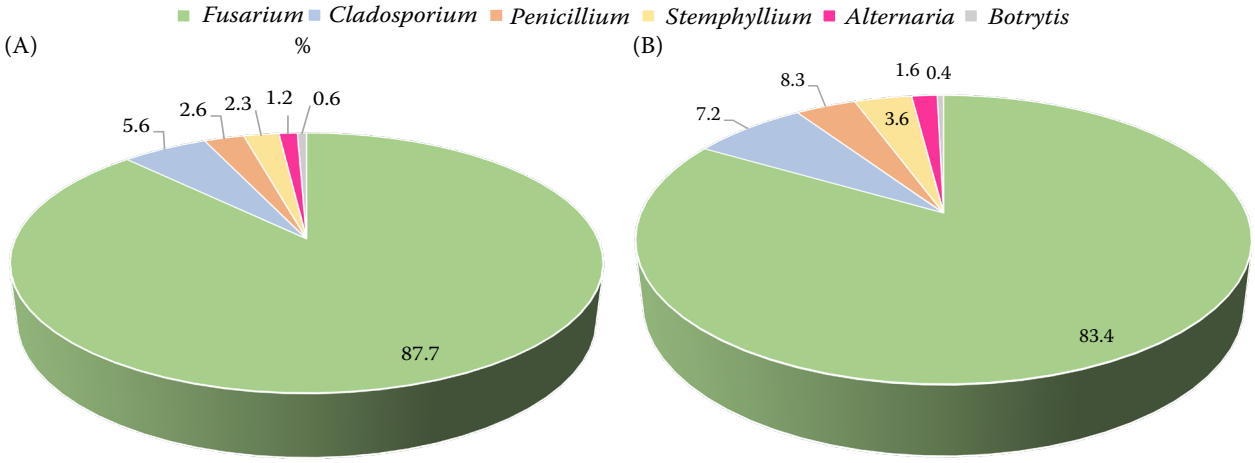

Figure 3. The percentage share of the individual genera of the fungi found in the green *Asparagus officinalis* spears of the different cultivars
(A) – with disease symptoms; (B) – without disease symptoms

The occurrence of species of the *Fusarium* genus depending on the spear fragment. A separate analysis of the isolates obtained from the fragments of the epidermis and parenchyma showed which species of the *Fusarium* genus were capable of colonising the deeper tissues of the green spears of the cultivars under study. In both groups of asparagus spears (with and without symptoms), the colonisation by *Fusarium* fungi was significantly higher in the epidermis than in the parenchyma. The following six species were identified among the isolates: *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. fujikuroi*. The parenchyma of the spears with disease symptoms

Table 2. Frequency of the isolates (%) of the species of the *Fusarium* genus in the green *Asparagus officinalis* spears of different cultivars (the mean value for the epidermis, parenchyma, for the harvest date, and three years of the research)

Cultivar	*F. culmorum*	*F. equiseti*	*F. oxysporum*	*F. proliferatum*	*F. solani*	*F. fujikuroi*
Symptom – no						
'Andreas'	0.0^{a}	0.0^{a}	0.8^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Ariane'	0.0^{a}	0.4^{a}	2.1^{bcd}	0.7^{a}	0.1^{a}	0.0^{a}
'Cipres'	0.5^{a}	1.0^{ab}	2.5^{cd}	0.5^{a}	0.5^{a}	0.4^{a}
'Eposs'	0.0^{a}	0.2^{a}	4.3^{e}	0.7^{a}	0.9^{ab}	0.1^{a}
'Grolim'	0.9^{ab}	1.3^{abc}	2.5^{cd}	1.3^{abc}	0.7^{a}	0.5^{a}
'Gynlim'	0.0^{a}	0.4^{a}	5.8^{f}	0.3^{a}	0.5^{a}	0.0^{a}
'Hannibal'	0.0^{a}	0.0^{a}	3.3^{de}	0.7^{a}	0.2^{a}	0.0^{a}
Symptom – yes						
'Andreas'	0.1^{a}	0.0^{a}	10.4^{d}	0.7^{a}	0.3^{a}	0.0^{a}
'Ariane'	0.0^{a}	1.3^{abc}	17.0^{f}	4.4c	0.7^{a}	0.5^{a}
'Cipres'	0.3^{a}	1.2^{abc}	13.0^{de}	0.7^{a}	0.3^{a}	0.4^{a}
'Eposs'	0.3^{a}	0.9^{ab}	17.9^{f}	4.2c	1.4^{abc}	0.4^{a}
'Grolim'	1.3^{abc}	1.3^{abc}	15.2^{e}	2.2^{abc}	0.4^{a}	0.8^{a}
'Gynlim'	0.2^{a}	0.9^{ab}	11.7^{d}	2.7^{abc}	0.7^{a}	0.0^{a}
'Hannibal'	0.3^{a}	0.0^{a}	15.3^{e}	3.2^{abc}	0.2^{a}	0.3^{a}
No symptom	0.2^{A}	0.5^{A}	3.1^{B}	0.6^{A}	0.4^{A}	0.1^{A}
Yes symptom	0.4^{A}	0.8^{A}	14.4^{C}	2.6^{B}	0.6^{A}	0.4^{A}

$^{a–f}$Means followed by the same letter in the column do not differ significantly at $\alpha = 0.05$

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was colonised by only two species, *F. oxysporum* and *F. proliferatum*. The spears without symptoms were colonised by *F. oxysporum* only (Table 3).

The occurrence of fungi of the *Fusarium* genus in the spears in the consecutive years of the study depending on the harvest date. In the first year of the study, *F. oxysporum* was the most abundant species found in the green *A. officinalis* spears with disease symptoms (Table 4).

There were statistically significant differences in the colonisation of the spears of the different cultivars by this fungus at the consecutive harvest dates. The other species were much less abundant. At the first harvest date, there were no *F. equiseti*, *F. proliferatum*, or *F. solani* fungi found on the green spears. *F. oxysporum* was the most abundant in the group of spears both with and without disease symptoms (Table 4). There was no *F. culmorum* or *F. proliferatum* at the first harvest date, and there were few *F. equiseti* and *F. solani* isolates.

In the second year of the study, there were statistically significant differences in the colonisation of the green *A. officinalis* spears with disease symptoms by *F. oxysporum* and *F. proliferatum* (Table 5).

Both species differed in the colonisation of the different cultivars at the consecutive harvest dates. At the first harvest date, there were no *F. proliferatum*, *F. solani*, or *F. fujikuroi* in the spears with disease symptoms. There were statistically significant differences in the colonisation of the spears by fungi of the *Fusarium* genus at the subsequent harvest dates. As far as the spears without disease symptoms are concerned, there were statistically significant differences between *F. oxysporum* and the other species (Table 5). At the first harvest date, there were no *F. proliferatum* or *F. fujikuroi* in the spears.

In the third year of the study, there were statistically significant differences between *F. oxysporum* and the other species in the colonisation of the green asparagus spears with disease symptoms (Table 6).

Table 3. Frequency of the isolates (%) of the species of the *Fusarium* genus in the green *Asparagus officinalis* spears of different cultivars in the epidermis and parenchyma (the mean value for three years of the research)

Cultivar	*F. culmorum*		*F. equiseti*		*F. oxysporum*		*F. proliferatum*		*F. solani*		*F. fujikuroi*	
	epidermis	parenchyma	epidermis	parenchyma	epidermis	parenchyma	epidermis	parenchyma	epidermis	parenchyma	epidermis	parenchyma
Symptom – no												
'Andreas'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	$1.5^{a–f}$	0.2^{ab}	0.0^{a}	$0.,0^{a}$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Ariane'	0.0^{a}	0.0^{a}	0.9^{abc}	0.0^{a}	2.9^{fg}	$1.1^{a–d}$	$1.3^{a–e}$	0.0^{a}	0.2^{ab}	0.0^{a}	0.0^{a}	0.0^{a}
'Cipres'	$1.1^{a–d}$	0.0^{a}	$2.0^{c–f}$	0.0^{a}	4.2^{gh}	$0.,9^{abc}$	$1.1^{a–d}$	0.0^{a}	$1.1^{a–d}$	0.0^{a}	0.9^{abc}	0.0^{a}
'Eposs'	0.0^{a}	0.0^{a}	0.4^{abc}	0.0^{a}	8.0^{i}	0.4^{abc}	$1.3^{a–e}$	0.0^{a}	$1.8^{b–f}$	0.0^{a}	0.2^{ab}	0.0^{a}
'Grolim'	$1.8^{b–f}$	0.0^{a}	2.7^{ef}	0.0^{a}	4.4^{h}	0.7^{abc}	2.7^{ef}	0.0^{a}	$1.3^{a–e}$	0.0^{a}	$1.1^{a–d}$	0.0^{a}
'Gynlim'	0.0^{a}	0.0^{a}	0.9^{abc}	0.0^{a}	9.1^{i}	2.4^{def}	0.7^{abc}	0.0^{a}	$1.1^{a–d}$	0.0^{a}	0.0^{a}	0.0^{a}
'Hannibal'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	4.9^{h}	$1.,8^{b–f}$	$1.3^{a–e}$	0.0^{a}	0.4^{abc}	0.0^{a}	0.0^{a}	0.0^{a}
Mean for epidermis: 1.5^{B}												
Mean for parenchymas: 0.2^{A}												
Symptom – yes												
'Andreas'	0.2^{ab}	0.0^{a}	0.0^{a}	0.0^{a}	15.5^{lm}	$5.3^{d–h}$	$1.3^{a–d}$	0.0^{a}	0.7^{abc}	0.0^{a}	0.0^{a}	0.0^{a}
'Ariane'	0.0^{a}	0.0^{a}	$2.7^{a–e}$	0.0^{a}	21.5^{no}	12.7^{kl}	$8.0^{g–j}$	0.9^{abc}	$1.3^{a–d}$	0.0^{a}	1.1^{abc}	0.0^{a}
'Cipres'	0.7^{abc}	0.0^{a}	$2.4^{a–e}$	0.0^{a}	18.7^{mn}	$7.3^{f–j}$	0.9^{abc}	0.4^{ab}	0.7^{abc}	0.0^{a}	0.9^{abc}	0.0^{a}
'Eposs'	0.7^{abc}	0.0^{a}	$1.8^{a–d}$	0.0^{a}	23.3^{o}	12.4^{kl}	$6.0^{e–i}$	$2.4^{a–e}$	$2.9^{a–e}$	0.0^{a}	0.9^{abc}	0.0^{a}
'Grolim'	$2.7^{a–e}$	0.0^{a}	$2.7^{a–e}$	0.0^{a}	20.9^{no}	9.5^{ijk}	$3.8^{a–f}$	0.7^{abc}	0.9^{abc}	0.0^{a}	$1.5^{a–d}$	0.0^{a}
'Gynlim'	0.4^{ab}	0.0^{a}	$1.8^{a–d}$	0.0^{a}	15.1^{lm}	8.2^{hij}	$4.7^{c–h}$	0.7^{abc}	$1.3^{a–d}$	0.0^{a}	0.0^{a}	0.0^{a}
'Hannibal'	0.7^{abc}	0.0^{a}	0.0^{a}	0.0^{a}	19.8^{no}	10.9^{jk}	$4.2^{b–g}$	$2.2^{a–e}$	0.4^{ab}	0.0^{a}	0.7^{abc}	0.0^{a}
Mean for epidermis: 4.6^{B}												
Mean for parenchymas: 1.7^{A}												

$^{a–n}$Means followed by the same letter in the same column do not differ significantly at $\alpha = 0.05$

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Table 4. Frequency of the isolates (%) of the species of the *Fusarium* genus in the green *Asparagus officinalis* spears of different cultivars at the subsequent harvesting dates (1., 2., 3.) in the first year (the mean value for the epidermis and parenchyma)

Cultivar	*F. culmorum*			*F. equiseti*			*F. oxysporum*			*F. proliferatum*			*F. solani*		
	1.	2.	3.	1.	2.	3.	1.	2.	3.	1.	2.	3.	1.	2.	3.
Symptom – no															
'Andrea'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Ariane'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	0.0^{a}	1.0^{ab}	3.0^{bcd}	3.0^{bcd}	0.0^{a}	1.0^{ab}	1.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}
'Cipres'	0.0^{a}	1.0^{ab}	2.0^{abc}	0.0^{a}	2.0^{abc}	2.0^{abc}	1.0^{ab}	2.0^{abc}	3.0^{bcd}	0.0^{a}	1.0^{ab}	0.0^{a}	1.0^{ab}	1.0^{ab}	1.0^{ab}
'Eposs'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	2.0^{abc}	3.0^{bcd}	4.0^{cde}	0.0^{a}	1.0^{ab}	0.0^{a}	0.0^{a}	2.0^{abc}	3.0^{bcd}
'Grolim'	0.0^{a}	2.0^{abc}	2.0^{abc}	1.0^{ab}	1.0^{ab}	3.0^{bcd}	1.0^{ab}	2.0^{abc}	4.0^{cde}	0.0^{a}	1.0^{ab}	1.0^{ab}	1.0^{ab}	1.0^{ab}	1.0^{ab}
'Gynlim'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	2.0^{abc}	5.0^{de}	6.0^{e}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Hannibal'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	2.0^{abc}	1.0^{ab}	0.0^{a}	0.0^{a}	1.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}
Mean for species: (1): 0.3^{A}, (2): 0.5^{A}, (3): 2.2^{B}, (4): 0.3^{A}, (5): 0.5^{A}															
Mean for harvesting dates (1.): 0.3^{A}, (2.): 0.9^{B}, (3): 1.1^{B}															
Symptom – yes															
'Andreas'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	4.0^{abc}	$14.0^{c–h}$	$14.0^{c–h}$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Ariane'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	3.0^{ab}	$8.0^{a–f}$	23.0^{hij}	29.0j	0.0^{a}	$6.0^{a–d}$	$9.0^{a–f}$	0.0^{a}	1.0^{a}	1.0^{a}
'Cipres'	0.0^{a}	1.0^{a}	1.0^{a}	0.0^{a}	2.0^{a}	0.0^{a}	3.0^{ab}	$13.0^{b–h}$	$20.0^{g–j}$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	$1.0.0^{a}$
'Eposs'	0.0^{a}	0.0^{a}	1.0^{a}	0.0^{a}	1.0^{a}	0.0^{a}	$10.0^{a–g}$	$15.0^{d–h}$	30.0	0.0^{a}	2.0^{a}	4.0^{abc}	0.0^{a}	2.0^{a}	4.0^{abc}
'Grolim'	1.0^{a}	3.0^{ab}	2.0^{a}	0.0^{a}	2.0^{a}	0.0^{a}	$7.0^{a–e}$	$18.0^{f–i}$	26.0^{ij}	0.0^{a}	3.0^{ab}	4.0^{abc}	0.0^{a}	1.0^{a}	0.0^{a}
'Gynlim'	1.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{a}	0.0^{a}	1.0^{a}	$15.0^{d–h}$	22.0^{hij}	0.0^{a}	4.0^{abc}	5.0^{abcd}	0.0^{a}	0.0^{a}	0.0^{a}
'Hannibal'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	$6.0^{a–d}$	$17.0^{e–i}$	26.0^{ij}	0.0^{a}	3.0^{ab}	3.0^{ab}	0.0^{a}	1.0^{a}	1.0^{a}
Mean for species: (1): 0.5^{A},(2): 0.5^{A},(3): 15.3^{B}, (4): 2.0^{A}, (5):0.6^{A}															
Mean for harvesting dates (1.): 1.2^{A}, (2.): 4.2^{B}, (3): 5.9^{C}															

$^{a–j}$Means followed by the same letter in the same column do not differ significantly at α = 0.05

There were also differences in the colonisation of the spears by this species in the cultivars at the consecutive harvest dates. At the first harvest date, there were no *F. culmorum*, *F. proliferatum, F. solani*, or *F. fujikuroi.* At the consecutive harvest dates, there were statistically significant differences in the occurrence of fungi of the *Fusarium* genus. *F. oxysporum* was the most abundant species in the spears without disease symptoms (Table 6). The following species were absent from the spears at the first date: *F. proliferatum* and *F. fujikuroi.* There was no *F. culmorum* at the second date. At the third date, *F. culmorum* and *F. solani* were absent from the spears.

The number of isolates of all the species of the *Fusarium* genus increased significantly in both groups of the spears since the second harvest date (Tables 5 and 6). The only exception was in the third year of the study in the spears without disease symptoms, when only the set from the third harvest date was distinguished.

The colonisation of *A. officinalis* spears by fungi of the *Fusarium* genus depending on the cultivar. The analysis of the mean colonisation of the green *A. officinalis* spears during the three years of the study showed that, in the group with disease symptoms, the colonisation level of the spears of the 'Eposs' and 'Ariane' cultivars was significantly higher than that of the other cultivars. In the group of spears without disease symptoms, the 'Grolim' and 'Gynlim' cultivars were distinguished by a higher percentage of *Fusarium* fungi and the difference was statistically significant (Figure 4).

Assessment of the pathogenicity of the selected isolates of the *Fusarium* genus against *A. officinalis* plants. The selected isolates of the *Fusarium* genus collected from different fragments of green spears differed in their pathogenicity against the *A. officinalis* plants (Figure 5). Among the *F. culmorum* isolates, the highest percentage of infected plants was found in the treatment with the isolate obtained from the outer part of the spears without disease symptoms – F.c./Mgr54. There were also differences in the degree of infection of the plants in the treatments where the *F. ox-*

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Table 5. Frequency of the isolates (%) of the species of the *Fusarium* genus in the green *Asparagus officinalis* spears of different cultivars at the subsequent harvesting dates (1., 2., 3.) in the second year (the mean value for the epidermis and parenchyma)

Cultivar	*F. culmorum*			*F. equiseti*			*F. oxysporum*			*F. proliferatum*			*F. solani*			*F. fujikuroi*		
	1.	2.	3.	1.	2.	3.	1.	2.	3.	1.	2.	3.	1.	2.	3.	1.	2.	3.
Symptom – no																		
'Andreas'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	2.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Ariane'	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	1.0^{ab}	0.0^{a}	0.0^{a}	3.0^{abc}	4.0^{abc}	0.0^{a}	1.0^{ab}	1.0^{ab}	0.0^{a}	1.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Cipres'	0.0^{a}	2.0^{ab}	0.0^{a}	0.0^{a}	2.0^{ab}	2.0^{ab}	1.0^{ab}	4.0^{abc}	5.0^{bc}	0.0^{a}	2.0^{ab}	2.0^{ab}	1.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	0.0^{a}
'Eposs'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	2.0^{ab}	0.0^{a}	4.0^{abc}	5.0^{bc}	5.0^{bc}	0.0^{a}	1.0^{ab}	2.0^{ab}	1.0^{ab}	1.0^{ab}	1.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}
'Grolim'	2.0^{ab}	0.0^{a}	0.0^{a}	1.0^{ab}	1.0^{ab}	2.0^{ab}	1.0^{ab}	4.0^{abc}	5.0^{bc}	0.0^{a}	3.0^{abc}	5.0^{bc}	2.0^{ab}	1.0^{ab}	0.0^{a}	0.0^{a}	2.0^{ab}	3.0^{abc}
'Gynlim'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	1.0^{ab}	4.0^{abc}	7.0 cd	10.0 d	0.0^{a}	1.0^{ab}	2.0^{ab}	1.0^{ab}	2.0^{ab}	2.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}
'Hannibal'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	5.0^{bc}	5.0^{bc}	0.0^{a}	2.0^{ab}	3.0^{abc}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Mean for species: (1): 0.2^{a}, (2): 0.7^{a}, (3): 3.6^{b}, (4): 1.2^{a}, (5): 0.6^{a}, (6): 0.3^{a}, (7):0.3^{a}																		
Mean for harvesting dates: (1): 0.5^{a}, (2): 1.3^{b}, (3): 1.5^{b}																		
Symptom – yes																		
'Andreas'	1.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	$7.0^{a–f}$	16.0^{ghi}	16.0^{ghi}	0.0^{a}	2.0^{abc}	$3.0^{a–d}$	0.0^{a}	1.0^{ab}	2.0^{abc}	0.0^{a}	0.0^{a}	0.0^{a}
'Ariane'	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	2.0^{abc}	2.0^{abc}	$6.0^{a–f}$	$14.0^{f–i}$	21.0^{ij}	0.0^{a}	$6.0^{a–f}$	$10.0^{c–g}$	0.0^{a}	1.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}	2.0^{abc}
'Cipres'	1.0^{ab}	0.0^{a}	0.0^{a}	1.0^{ab}	1.0^{ab}	$3.0^{a–d}$	$6.0^{a–f}$	17.0^{ghi}	20.0^{ij}	0.0^{a}	0.0^{a}	1.0^{ab}	0.0^{a}	1.0^{ab}	0.0^{a}	0.0^{a}	1.0^{ab}	1.0^{ab}
'Eposs'	1.0^{ab}	1.0^{ab}	0.0^{a}	0.0^{a}	2.0^{abc}	$3.0^{a–d}$	$12.0^{e–h}$	16.0^{ghi}	26.0^{j}	0.0^{a}	$11.0^{d–g}$	$14.0^{f–i}$	0.0^{a}	1.0^{ab}	1.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}
'Grolim'	2.0^{abc}	1.0^{ab}	0.0^{a}	1.0^{ab}	2.0^{abc}	2.0^{abc}	$7.0^{a–f}$	20.0^{ij}	22.0^{ij}	0.0^{a}	$4.0^{a–e}$	$6.0^{a–f}$	0.0^{a}	1.0^{ab}	2.0^{abc}	0.0^{a}	2.0^{abc}	1.0^{ab}
'Gynlim'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	2.0^{abc}	1.0^{ab}	$4.0^{a–e}$	16.0^{ghi}	19.0^{hij}	0.0^{a}	$5.0^{a–e}$	$7.0^{a–f}$	0.0^{a}	2.0^{abc}	2.0^{abc}	0.0^{a}	0.0^{a}	0.0^{a}
'Hannibal'	1.0^{ab}	2.0^{abc}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	$9.0^{b–g}$	17.0^{ghi}	25.0^{j}	0.0^{a}	$7.0^{a–f}$	$6.0^{a–f}$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Mean for species: (1): 0.5^{a}, (2): 1.1^{a}, (3): 15.0^{c}, (4): 3.9^{b}, (5): 0.7^{a}, (6): 0.3^{a}																		
Mean for harvesting dates: (1): 1.4^{a}, (2): 4.1^{b}, (3): 5.2^{b}																		

$^{a–j}$Means followed by the same letter in the same column do not differ significantly at $\alpha = 0.05$

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Table 6. Frequency of the isolates (%) of the species of the *Fusarium* genus in the green *Asparagus officinalis* spears of different cultivars at the subsequent harvesting dates (1., 2., 3.) in the third year (the mean value for the epidermis and parenchyma)

Cultivar	*F. culmorum*			*F. equiseti*			*F. oxysporum*			*F. proliferatum*			*F. solani*			*F. fujikuroi*		
	1.	2.	3.	1.	2.	3.	1.	2.	3.	1.	2.	3.	1.	2.	3.	1.	2.	3.
Symptom – no																		
'Andreas'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	$3.0^{a–d}$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Ariane'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	0.0^{a}	0.0^{a}	0.0^{a}	$5.0^{a–d}$	0.0^{a}	0.0^{a}	2.0^{abc}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Cipres'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0 ab	0.0^{a}	$3.0^{a–d}$	$4.0^{a–d}$	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	0.0^{a}	0.0^{a}	1.0^{ab}	2.0^{abc}
'Eposs'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	$4.0^{a–d}$	$5.0^{a–d}$	7.0^{cde}	0.0^{a}	2.0^{abc}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{ab}	0.0^{a}
'Grolim'	2.0^{abc}	0.0^{a}	0.0^{a}	0.0^{a}	$3.0^{a–d}$	0.0^{a}	0.0^{a}	0.0^{a}	$6.0^{b–e}$	0.0^{a}	2.0^{abc}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Gynlim'	0.0^{a}	0.0^{a}	0.0^{a}	2.0^{abc}	0.0^{a}	0.0^{a}	$3.0^{a–d}$	$4.0^{a–d}$	11.0^{e}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Hannibal'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	7.0^{cde}	8.0^{de}	0.0^{a}	0.0^{a}	0.0^{a}	2.0^{abc}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Mean for species: (1): 0.1^{a}, (2): 0.3^{a}, (3): 3.3^{b}, (4): 0.3^{a}, (5): 1.0^{a}, (6): 2.0^{a}																		
Mean for harvesting dates: (1): 0.3^{a}, (2): 0.7^{a}, (3): 1.1^{b}																		
Symptom – yes																		
'Andreas'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	3.0^{a}	7.0^{ab}	13.0^{bc}	0.0^{a}	1.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Ariane'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	2.0^{a}	2.0^{a}	5.0^{a}	23.0^{fgh}	25.0^{gh}	0.0^{a}	5.0^{a}	4.0^{a}	0.0^{a}	2.0^{a}	1.0^{a}	0.0^{a}	2.0^{a}	1.0^{a}
'Cipres'	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}	3.0^{a}	13.0^{bc}	$22.0^{e–h}$	0.0^{a}	3.0^{a}	2.0^{a}	0.0^{a}	0.0^{a}	1.0^{a}	0.0^{a}	1.0^{a}	1.0^{a}
'Eposs'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{a}	1.0^{a}	5.0^{a}	$20.0^{d–g}$	27.0^{h}	0.0^{a}	4.0^{a}	3.0^{a}	0.0^{a}	3.0^{a}	2.0^{a}	0.0^{a}	2.0^{a}	2.0^{a}
'Grolim'	0.0^{a}	1.0^{a}	2.0^{a}	2.0^{a}	1.0^{a}	2.0^{a}	4.0^{a}	16.0^{cde}	$17.0^{c–f}$	0.0^{a}	2.0^{a}	1.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{a}	3.0^{a}
'Gynlim'	0.0^{a}	1.0^{a}	0.0^{a}	1.0^{a}	1.0^{a}	2.0^{a}	2.0^{a}	6.0^{a}	$20.0^{d–g}$	0.0^{a}	2.0^{a}	1.0^{a}	0.0^{a}	2.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
'Hannibal'	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	3.0^{a}	15.0^{cd}	$20.0^{d–g}$	0.0^{a}	5.0^{a}	5.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	1.0^{a}	2.0^{a}
Mean for species: (1): 2.0^{a}, (2): 0.8^{ab}, (3): 12.8^{c}, (4): 1.8^{b}, (5): 0.5^{ab}, (6): 0.8^{ab}																		
Mean for harvesting dates: (1): 0.7^{a}, (2): 3.4^{b}, (3): 4.3^{b}																		

$^{a–h}$Means followed by the same letter in the same column do not differ significantly at $\alpha = 0.05$

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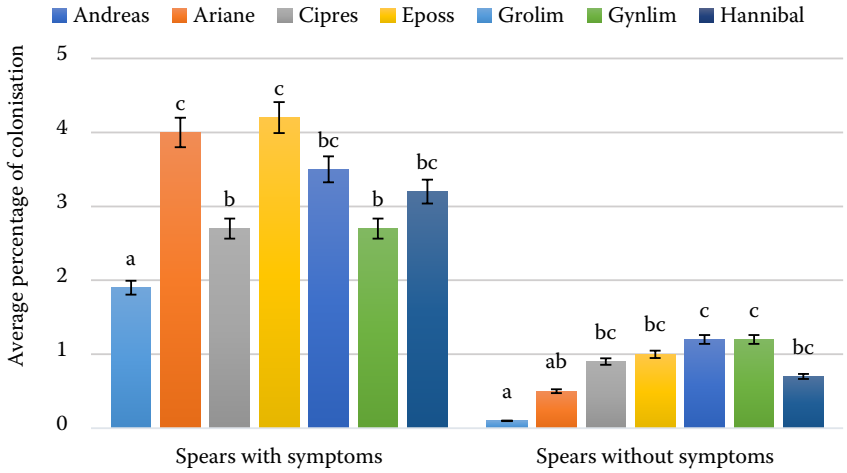

Figure 4. The occurrence of the species of the *Fusarium* genus in the green *Asparagus officinalis* spears depending on the cultivar (mean for three years, for the epidermis and parenchyma)
Means followed by the same letter do not differ significantly at α = 0.05

ysporum isolates were used. The highest degree was observed in the treatments with the F.o./Mar7 isolate collected from the epidermis of the spears with disease symptoms. In the treatments where the pathogenicity of *F. proliferatum* isolates was assessed, the Fp/Mar3 and Fp/Mar1 isolates showed the highest percentage of infected plants. There were no statistically significant differences in the pathogenicity in the treatments with the *F. equiseti*, *F. solani*, and *F. fujikuroi* isolates.

DISCUSSION

The analysis of the composition of the fungi isolated from the green *A. officinalis* spears showed that most of the isolates belonged to the *Fusarium* genus. Fungi of other species (*Alternaria*, *Botrytis*, *Cladosporium*, *Penicillium*, and *Stemphylium*) were isolated sporadically. The following six species of fungi of the *Fusarium* genus were isolated from the *Asparagus officinalis* spears: *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. verticillioides*. *F. oxysporum* was the dominant species in the spears under analysis. Other researchers also analysed the colonisation of asparagus spears with fungi of the *Fusarium* genus. Elmer (2000) examined 67 samples collected from both production and experimental plantations in the states of Connecticut, Washington, California, and North Carolina, as well as Mexico and Peru. The spears were available in different months, depending on the harvest time in the area they came from, where the isolates were collected from the base and apex. The spears under analysis had no disease symptoms. The study by this author showed that 23% of the spears were colonised by fungi of the *Fusarium* genus, including *F. proliferatum* isolates (53%), *F. oxysporum* isolates (30%), and a few *F. fujikuroi*, *F. subglutinans*, *F. solani*, *F. acuminatum*, *F. graminearum*, *F. semi-*

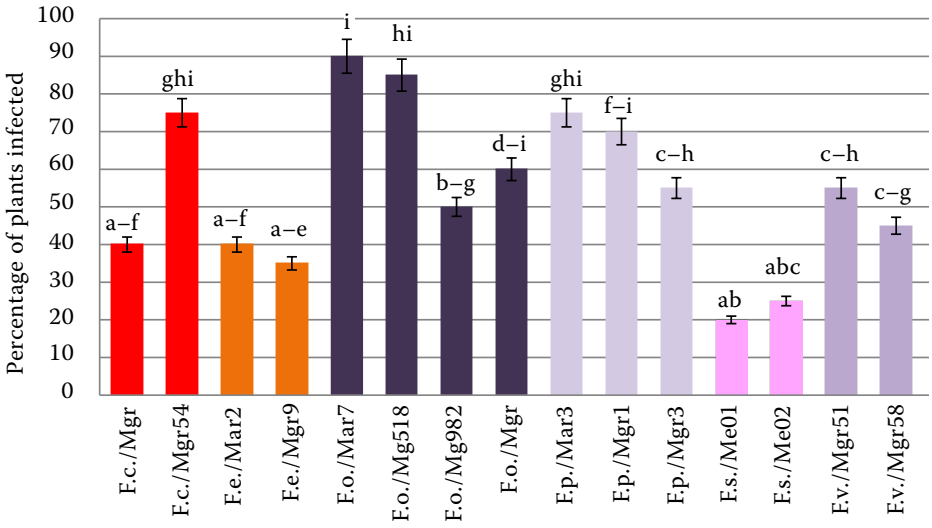

Figure 5. The pathogenicity of the fungi of the *Fusarium* genus against *Asparagus officinalis* plants in the growth chamber
Means followed by the same letter do not differ significantly at α = 0.05

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tectum, and *F. culmorum* isolates (17%). All these species were isolated from the spear apex. *F. proliferatum* was most often isolated from the base, which was particularly noticeable in the material collected from North Carolina, Mexico, and Peru. The base of all these spears was more strongly colonised than the apex. Gossman et al. (2005) made a more detailed analysis of the distribution of fungi in various parts of asparagus spears collected from plantations in Germany. The researchers took both external and internal tissues into account. They found that *F. proliferatum* was not a rare species in this material. The fungi usually colonised the spears asymptomatically, but this species was also isolated from the base of a spear with orange and grey discoloration. *F. oxysporum* isolates were the most abundant, followed by *F. subglutinans*, *F. redolens*, *F. merismoides*, *F. equiseti*, *F. lateritium*, and *F. dimerum*. The comparison of the results of the studies by Gossman et al. (2005) and Vujanović et al. (2006) concerning the geographical distribution of individual species of the *Fusarium* genus showed that *F. oxysporum* was the dominant species in both the material from Germany and Canada. As far as other isolates are concerned, *F. lateritium* and *F. dimerum* were more prevalent in colder regions of Canada, whereas *F. redolens* and *F. proliferatum* were more often found in warmer regions. Weber et al. (2007) made similar analyses in Poland. The researchers assessed the health status of blanched and green asparagus spears harvested in 2005 and 2006. They separately analysed the spears with and without disease symptoms. The research showed that *F. oxysporum* and *F. proliferatum* were the most common isolates of the *Fusarium* genus, whereas *F. culmorum*, *F. heterosporum*, *F. scripi*, *F. solani*, and *F. fujikuroi* were less common. The researchers also observed that the abundance of isolates largely depended on the part of the spear (base, apex, centre, external or internal layer) from which the material was collected and the presence of discoloration on the surface of the epidermis. Like Elmer (2000) and Gossman et al. (2005) noted, the base of the spears, especially those with symptoms, was the most heavily colonised by fungi. The results of our research are in line with the findings of the study by Weber et al. (2007). The detailed analysis also showed that isolations from the spears with visible discolorations and harvested at the latest date resulted in the most numerous and the most representative group of isolates of various species of the *Fusarium* genus. The harvest date significantly influenced the count of the isolates. The highest counts were isolated at the late harvest period.

As in the study by Weber et al. (2007), our research also showed that the green spears were less colonised by fungi of the *Fusarium* genus during the harvest period. Apart from that, our study revealed a significant correlation between the harvest date and the number of isolates as well as the presence of various species of the *Fusarium* genus in the spears. This correlation is important not only for the assessment of the quality of spears, but it should also be taken into account in studies assessing the susceptibility of the cultivars to diseases or the pathogenicity of isolates. The results of these studies should be compared with the results of similar observations made by other researchers.

Our assessment of the health of the green spears of various *A. officinalis* cultivars revealed cultivar-dependent differences in the intensity of the fungal colonisation. Vujanović et al. (2006) indicated the importance of cultivars in research on the population of fungi of the *Fusarium* genus. They noticed that the number of isolates was smaller on plantations with the 'Guelph Millennium' cultivar. They also indicated that the 'Mary Washington' cultivar, which is commonly grown in the United States and was grown in Europe until 2000, was susceptible to fungal colonisation. Currently, there is a wide range of cultivars grown in Poland and other European countries. Gossman et al. (2005) observed differences in the colonisation of asparagus spears of different cultivars by fungi of the *Fusarium* genus. The lowest fungal colonisation was noted in the 'Gijnlim' and 'Ariane' cultivars, whereas the colonisation of the 'Grolim', 'Andreas', and 'Eposs' cultivars was average. In our study, 'Eposs' and 'Ariane', as well as 'Grolim' and 'Gynlim', were the cultivars with the highest populations of fungi of the *Fusarium* genus. The external tissues of the spears of these cultivars were characterised by particularly high fungal colonisation. These authors suggested that saprotrophic isolates of the *Fusarium* genus, which can colonise plant tissues, may come from plants without noticeable disease symptoms. It cannot be ruled out that, among the several hundred isolates identified during the observations, there were also non-pathogenic ones. However, the infection tests showed that the pathogenic isolates of all the species were obtained from both the external and internal tissues of the spears, both with and without disease symptoms. Apart from that, the isolates analysed differed significantly in the pathogenicity in our

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study. This difference was observed both between the different species and within the isolates of the same species. Vujanović et al. (2006) analysed the sequence of the EF-1 alpha gene and found high similarity (up to 99.76%) between some *F. proliferatum* isolates originating from Quebec and the US. However, the molecular analysis also revealed significant diversity in a large pool of isolates – it was smaller among the *F. proliferatum* isolates, but significantly greater among the *F. oxysporum* isolates. The EF-1 alpha gene is not related to the pathogenicity, but there is considerable variation within the isolates of the same species both in terms of this trait and other traits.

According to data provided in the reference publications, as well the results of our observations, there are numerous factors which may modify the populations of fungi of the *Fusarium* genus on *A. officinalis* plantations. Pathogenic species of the *Fusarium* genus as well as the changing spectrum of cultivars are extremely important and constantly changing elements affecting the cultivation of asparagus. For this reason, the constant monitoring and assessment of the health of asparagus plants growing on plantations are extremely important for horticultural practice.

CONCLUSIONS

The analysis of the composition of the fungi isolated from the green *Asparagus officinalis* spears showed that most of the isolates belonged to the *Fusarium* genus (*F. culmorum, F. equiseti, F. oxysporum, F. proliferatum, F. solani*, and *F. fujikuroi*). Other fungal species (*Alternaria, Botrytis, Cladosporium*, *Penicillium*, and *Stemphylium*) were rarely isolated. The assessment of the health of the green spears of different *A. officinalis* cultivars showed cultivar-dependent differences in the fungal colonisation. Most of the *Fusarium* genus isolates came from the spears of the 'Ariane' and 'Eposs' cultivars with disease symptoms and from the spears of the 'Grolim' cultivar without disease symptoms. The *Fusarium* genus fungi colonised both the spears with and without disease symptoms, but there were always more isolates on the ones with disease symptoms. The count of the isolates also depended on the spear fragment. The *Fusarium* genus fungi occurred more often in the epidermis than in the parenchyma. *F. oxysporum* was the dominant fungus in the *A. officinalis* spears under analysis. The number of isolates of the fungi of the *Fusarium* genus collected from the green *A. officinalis* spears tended to increase at the consecutive harvest dates. This means that the spears harvested at the latest date (late June) were the most heavily colonised by fungi. All the isolates of the fungi of the *Fusarium* genus collected from the spears exhibited pathogenicity against *A. officinalis* plants.

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