

## The occurrence of *Fusarium* species in white spears of *Asparagus officinalis*

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### Abstract

The aim of the study was to determine the species composition of fungi of the *Fusarium* genus found on white *Asparagus officinalis* spears, depending on the presence or absence of disease symptoms, age of the plantation, date of harvest and the place from which samples were collected for mycological analysis. Additionally, the pathogenicity of selected *Fusarium* spp. isolates was determined. *A. officinalis* L. was cultivated for white spears. The research was conducted on the German dioecious cultivar 'Eposs'. Samples of *A. officinalis* spears for tests were collected from two plantations. Six species of fungi of the *Fusarium* genus were identified in the asparagus spears: *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. fujikuroi*. Among the *Fusarium* species colonizing *Asparagus officinalis* the greatest threat is *F. oxysporum*. Always there are more isolates in the spears with the symptoms, in epidermis. The late harvest date favors the development of fusariosis. This means that the spears harvested at the latest date (late June) are the most heavily colonised by fungi. The isolates of fungi of the *Fusarium* genus collected from the spears exhibit pathogenicity against *A. officinalis* plants.

**Keywords:** asparagus spears; epidermis; *Fusarium*; parenchyma; plantation age

### Introduction

Numerous *Asparagus officinalis* L. cultivars have a prominent position among the field vegetables which are harvested very early. Young, juicy shoots known as spears are the edible part of asparagus, which is valued for its taste and high nutritional value. According to the Food and Agriculture Organisation (FAO) (2019), the world production of *A. officinalis* is increasing year by year. In 2008 the yield was estimated at 7,215,844 tonnes, whereas in 2019 it amounted to 9,432,062 tonnes. China is the largest asparagus producer in the world – in 2019 the yield amounted to 8,306,073 tonnes. The total yield of *A. officinalis* in the European Union ranged from 255,324 tonnes in 2008 to 320,003 tonnes in 2019. In North and South Americas asparagus is grown mainly for green spears (Benson, 2008), whereas in Europe white spears is more widespread. Germany is the largest asparagus producer in Europe. Recently the area of *A. officinalis* plantations in Poland has also been increasing. More than a half of the production volume is exported. *A. officinalis* is becoming more and more popular on the Polish market. White spears are the most popular, but the demand for green ones is also increasing. *A. officinalis* spears growing on Polish plantations are harvested in May and June (Knaflewski *et al.*, 2014).

Received: 04 Nov 2021. Received in revised form: 13 Dec 2021. Accepted: 13 Dec 2021. Published online: 20 Dec 2021.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Fungal diseases are a problem in the cultivation of *A. officinalis*. Fungal pathogens such as *Puccinia asparagi*, *Botrytis cinerea*, and *Stemphylium botryosum* damage *A. officinalis* shoots and branches. Various species of the *Fusarium* genus cause shoot base and root rot as well as the wilting and dieback of plants (Baayen, *et al*; 2000). The following fungal species are most often isolated from *A. officinalis* shoots and crowns: *Fusarium oxysporum* Schlechtendahl emend. Snyder et Hansen, *F. culmorum* (W.G. Smith) Saccardo, *F. proliferatum* (Matsushima) Nirenberg, and *F. fujikuroi* Nirenberg. These fungi may also cause the discoloration and rust on *A. officinalis* spears, which lower the crop quality. Some species of the *Fusarium* genus produce secondary metabolites toxic to humans (Nesic *et al.*, 2013). Fungi of the *Fusarium* genus, which are commonly found in soil, either parasitise living plants or feed on their debris (saprotrophs). These fungi cause great damage because they produce numerous conidia, which help them spread with wind and rain in the natural environment. In consequence, very resistant spores (chlamydoconidia) are formed in soil, on plant debris and the propagation material, thanks to which they can survive long-lasting unfavourable conditions (Tuszyńska, 2010).

The establishment and maintenance of an *A. officinalis* plantation involves high costs. The first crops can be harvested no sooner than in the second year. The yield increases in subsequent years, so the depreciation costs decrease as the length of the harvest time increases. The expected operating time of an *A. officinalis* plantation is ten years. The quality of asparagus spears is equally important, because their prices vary widely and the income depends more on the crop quality rather than yield. For these reasons, fungi of the *Fusarium* genus pose a significant threat to the yield quality. The life of plantations infested with fungi is shorter, whereas *A. officinalis* spears are small and deformed. The occurrence and damage caused by fungi of the *Fusarium* genus to asparagus plantations were described by researchers from the Netherlands (Blok and Bollen, 1995), Switzerland (Gordon-Lennox and Gindrat, 1987), Germany (Gossmann *et al.*, 2001), Italy (Tomassoli *et al.*, 2007), Spain (Wong and Jeffries, 2006), and Poland (Sadowski, 1981; Knaflewski *et al.*, 1993; Weber and Knaflewski, 2004). The problem of fusariosis on *A. officinalis* was also reported by scientists in Asia (Tu, 1979), Australia, New Zealand (Falloon and Tate, 1986; Elmer *et al.*, 1997), the US (Hartung *et al.*, 1990), Canada (Caron *et al.*, 1985), South America, and South Africa (Schreuder *et al.*, 1995; Quilambaqui-Jara, 2004). Regardless of the region in which research was conducted, several different species of the *Fusarium* genus were isolated from diseased *A. officinalis* plants. The fungi were isolated from various organs of plants of different ages: rhizomes, roots, shoots, and spears. Fungal diseases were manifested with symptoms such as seedling dieback, shoot yellowing and dieback (van Bakel and Krom-Kerstens, 1974). The presence of *Fusarium* fungi on rhizomes was manifested by initially red and brown irregularly-shaped and sized spots, which later developed into more visible lesions, whereas spears infested with fungi had rusty spots (van Bakel and Krom-Kerstens, 1974; Cheah, 1986, Hartmann, 1989).

The aim of the study was to determine the species composition of fungi of the *Fusarium* genus found on white *A. officinalis* spears of the 'Epos' cultivar, depending on the presence or absence of disease symptoms, age of the plantation, date of harvest and the place from which samples were collected for mycological analysis. Additionally, the pathogenicity of selected *Fusarium* spp. isolates was determined.

## Materials and Methods

### *Study site and biological material*

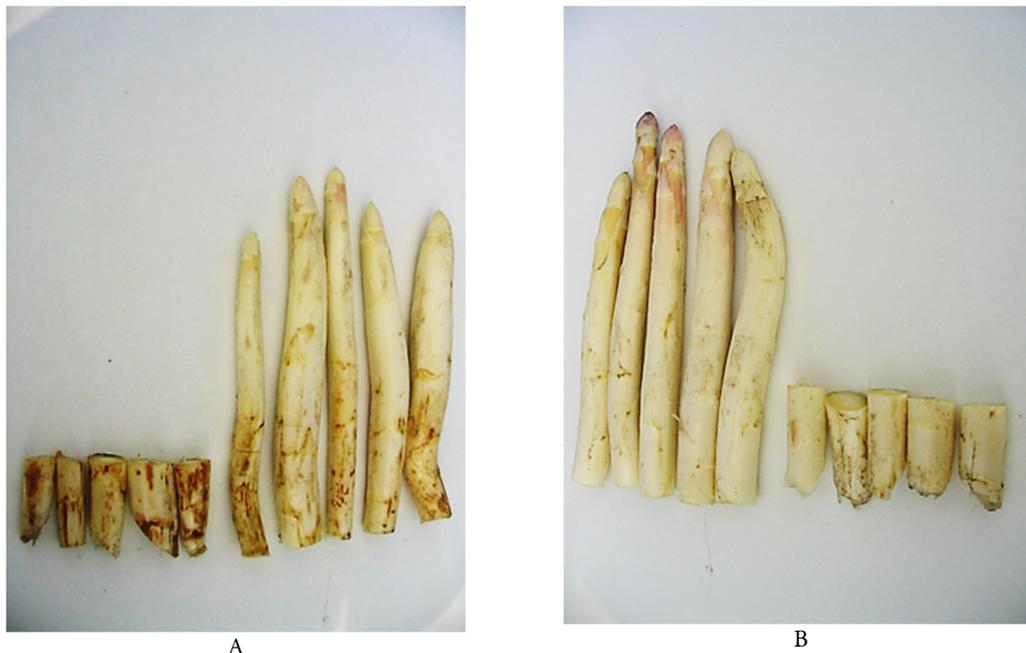
The research was conducted on a Polish plantation in Świdwowiec (52°22'18" N 15°58'49" E), Lubuskie Voivodeship, which was observed for three years for the occurrence of fungi of the *Fusarium* genus. The plantation was established on lessive soil, valuation class VI, which was totally composed of sand. *A. officinalis* L. was cultivated for white spears. The research was conducted on the German dioecious cultivar 'Eposs'. Samples of *A. officinalis* spears for tests were collected from two plantations:

I– the older plantation – in the seventh, eighth, and ninth years of cultivation;

II– the younger plantation (established three years later) – in the fourth, fifth, and sixth years of cultivation.

In the consecutive years of the research 10 spears with visible disease symptoms at the base (brown irregularly-shaped stains or rusty spots) and 10 spears without disease symptoms were randomly collected from each plot three times during the harvest period (on 1 and 25 May, and 19 June) (Figure 1).

The white spears with and without visible disease symptoms were analysed mycologically for the presence of fungi of the *Fusarium* genus. Fragments for the analysis were collected from the epidermis and parenchyma. The same procedure was applied to analyse the spears collected at different harvest dates from plantations I and II.



**Figure 1.** White *A. officinalis* 'Eposs' spears. A – with disease symptoms, B – without disease symptoms

The spears were washed with tap water and dried on tissue paper. Then they were visually inspected 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 outer part (epidermis) of each spear as well as the inner part (parenchyma) located directly below the epidermis. Next, the fragments collected from each spear were placed in Petri dishes with glucose and potato agar (PDA Merck). 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 and Knaflewski, 2004). After solidification the inoculum (5 epidermis and 5

parenchyma fragments from each base) 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 solidified Potato Dextrose Agar (PDA) agar in test tubes. The reinoculation resulted in cultures of the same age, which were segregated into homogenous groups, characterised macroscopically and microscopically, and identified according to mycological keys (Kwaśna *et al.*, 1991; Leslie and Summerell, 2006). A group of isolates was selected for further investigations from the resulting cultures of different species.

The Statistica 8.0 software was used for statistical calculations based on a three-way analysis of variance, which was conducted separately for both groups under study (spears with and without disease symptoms).

The following categories were assessed:

-the occurrence of various fungal species of the *Fusarium* genus in white *A. officinalis* 'Eposs' spears (the mean value for the epidermis and parenchyma). The following factors were analysed in the experiment: A – the three years of observation, B – fungal species (*F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. fujikuroi*); C – the age of the plantation;

-the occurrence of various fungal species of the *Fusarium* genus in the epidermis and parenchyma of white *A. officinalis* 'Eposs' spears. The following factors were analysed in the experiment: A – the part of the spear (epidermis, parenchyma), B – fungal species (*F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. fujikuroi*); C – the age of the plantation;

-the occurrence of various fungal species of the *Fusarium* genus in white *A. officinalis* 'Eposs' spears depending on the harvest date. The following factors were analysed in the experiment: A – the harvest date (1, 2, 3), B – fungal species (*F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. fujikuroi*); C – the age of the plantation.

One-way analysis of variance was applied to assess the colonisation of white *A. officinalis* spears by fungi of the *Fusarium* genus depending on the age of the plantation, the mean values of fungal colonisation of the epidermis and parenchyma of the spears, and the three years of observation. The one-way ANOVA was conducted separately for both groups under study (spears with and without disease symptoms).

The pathogenicity of selected isolates of the *Fusarium* genus was assessed in experiments conducted in a growth chamber. The pathogenicity of isolates collected from white spears with and without disease symptoms was assessed. The group of isolates of various species of the *Fusarium* genus included items collected from the epidermis and the spear parenchyma. The list of selected isolates is given in Table 1. The pathogenicity of selected isolates was assessed under controlled conditions. The experiment was conducted twice.

The research was conducted on the plants which developed from commercially available seeds of *A. officinalis* 'Andreas'. Before the seeds were placed on sprouters, they had been disinfected in flasks containing acetone with 5% Benlate 50 WP. Next, the whole 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.

**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
<i>F. culmorum</i>	F.c./Se971	+			
	F.c./Se972		+		
	F.c./Se9754				+
	F.c./Se9758			+	
<i>F. equiseti</i>	F.e./Se9712	+			
	F.e./Se9761		+		
	F.e./Se9769			+	
	F.e./Se978				+
<i>F. oxysporum</i>	F.o./Se971	+			
	F.o./Se972		+		
	F.o./Se973			+	
	F.o./Se0511				+
<i>F. proliferatum</i>	F.p./Se02		+		
	F.p./Se053	+			
	F.p./Se971			+	
	F.p./Se972				+
<i>F. solani</i>	F.s./Se976	+			
	F.s./Se9752			+	
<i>F. fujikuroi</i>	F.v./Se9752			+	
	F.v./Se9757	+			

*Experimental conditions*

The pots with the plants (220) 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 illuminated cyclically for 12 hours throughout the entire period. The observations were terminated after 16 weeks. During the experiment the plants were regularly inspected every 3 days. Isolates were collected from the dying plants on the PDA medium (Merck). On the last day of cultivation isolates were collected from all the other plants. They were cleaned from the remnants of the substrate. Next, 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 Petri dishes. The dishes were incubated in a thermostat cabinet at 23 °C for 14 days. During this time the emerging cultures of fungi were split off and their species were identified on the basis of macro- and microscopic traits.

There were 22 treatments of the growth chamber experiment, each with 10 replications:

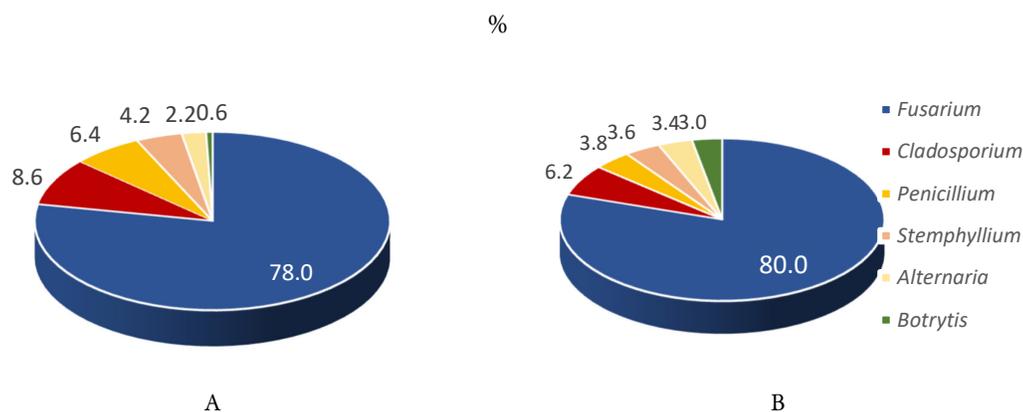
1. control variant A – absolute control (substrate without inoculum)
2. control variant B – methodical control (substrate with a disc of solidified PDA medium)
- 3 to 6 – substrate with an inoculum containing various *F. culmorum* isolates
- 7 to 10 – substrate with an inoculum containing various *F. equiseti* isolates
- 11 to 14 – substrate with an inoculum containing various *F. oxysporum* isolates
- 15 to 18 – substrate with an inoculum containing various *F. proliferatum* isolates
- 19 to 20 – substrate with an inoculum containing various *F. solani* isolates
- 21 to 22 – substrate with an inoculum containing various *F. fujikuroi* isolates

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

## Results

### *The occurrence of fungi in white A. officinalis spears of the 'Eposs' cultivar*

During the three years of the research a total of 3,600 fragments were collected from the white *A. officinalis* spears for mycological analysis (half of them from the spears with disease symptoms and the other half without symptoms). The research material was assessed for the occurrence of fungi. The fungal species were identified and their populations at the plots/sites of different ages and at individual harvest dates were determined. The fungi of the *Fusarium* genus were the most numerous groups of isolates (78% - 1404 and 80% - 1440) found in the *A. officinalis* spears. Representatives of the *Alternaria*, *Botrytis*, *Cladosporium*, *Penicillium*, and *Stemphyllium* genera were also identified. The share of these isolates in the spears with and without disease symptoms amounted to 22% and 20%, respectively (Figure 2AB).



**Figure 2.** The percentage share of individual genera of fungi found in white *A. officinalis* 'Eposs' spears (the mean value for both plantations during three years of the research)

A – with disease symptoms, B – without disease symptoms.

### *The isolated species of the Fusarium genus and their counts*

The following six species of the *Fusarium* genus were isolated from the *A. officinalis* spears: *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. fujikuroi*. There were statistically significant differences in the colonisation of the spears with disease symptoms by individual species of the *Fusarium* genus (Table 2).

The most numerous species were *F. oxysporum* and *F. proliferatum*. There were also significant differences in the colonisation of the asparagus spears by the same fungus species depending on the age of the plantation. There were more isolates found in the spears from the older plantation. The differences were particularly marked for *F. oxysporum*, *F. proliferatum*, and *F. equiseti* (Table 2).

In the group of spears without disease symptoms there were statistically significant differences in the count of *F. oxysporum* isolates, which were the most numerous, and the other species of the *Fusarium* genus. There were no significant differences between the older (I) and younger (II) plantations in the colonisation of the spears by fungi of the *Fusarium* genus (Table 3).

**Table 2.** The occurrence of species the *Fusarium* genus in white *A. officinalis* 'Eposs' spears (the mean value for the epidermis, parenchyma, and three years of the research)

Species	Plantation	Frequency of isolates (%) in spears			
		With symptoms		Without symptoms	
		Mean		Mean	
<i>F. culmorum</i>	I	3.1 ab	1.7 ab	1.9 a	1.2 a
	II	0.3 a		0.4 a	
<i>F. equiseti</i>	I	5.2 b	3.3 bc	1.4 a	1.1 a
	II	1.4 a		0.8 a	
<i>F. oxysporum</i>	I	32.9 e	30.5 d	11.7 b	11.5 b
	II	28.2 d		11.3 b	
<i>F. proliferatum</i>	I	8.4 c	5.9 c	1.5 a	0.9 a
	II	3.3 ab		0.3 a	
<i>F. solani</i>	I	1.8 ab	0.9 ab	0.3 a	0.2 a
	II	0.0 a		0.0 a	
<i>F. fujikuroi</i>	I	1.3 a	0.7 a	0.4 a	0.3 a
	II	0.1 a		0.1 a	

Means followed by the same letter do not differ significantly at  $\alpha = 0.05$

*The occurrence of species of the Fusarium genus depending on the spear fragment*

There were significant differences in the count of isolates found in the epidermis and the parenchyma of the spears in both groups (with and without disease symptoms). The outer part (epidermis) of the spears had more fungi of the *Fusarium* genus than the inner part (parenchyma) (Table 3). *F. oxysporum* isolates were the dominant group. Their count was significantly greater than that of the other species. Moreover, in the group of spears with disease symptoms there were significant differences in the colonisation of the epidermis by the *F. culmorum*, *F. equiseti*, and *F. proliferatum* species depending on whether the spears came from the older or younger plantation. These species were more abundant in the older plantation. These differences were not observed in the group of spears without disease symptoms (Table 3).

**Table 3.** The occurrence of species the *Fusarium* genus in the epidermis and parenchyma of white *A. officinalis* 'Eposs' spears (the mean value for three years of the research)

Species	Plantation	Frequency of isolates (%) in spears			
		With symptoms		Without symptoms	
		Epidermis	Parenchyma	Epidermis	Parenchyma
<i>F. culmorum</i>	I	5.5 bc	0.7 a	3.3 a	0.4 a
	II	0.7 a	0.0 a	0.9 a	0.0 a
<i>F. equiseti</i>	I	7.8 c	2.7 ab	2.7 a	0.2 a
	II	2.7 ab	0.2 a	1.5 a	0.0 a
<i>F. oxysporum</i>	I	38.2 g	27.5 f	15.5 c	7.8 b
	II	34.2 g	22.2 e	14.2 c	8.4 b
<i>F. proliferatum</i>	I	12.2 d	4.7 abc	2.9 a	0.2 a
	II	4.4 abc	2.2 ab	0.7 a	0.0 a
<i>F. solani</i>	I	3.5 abc	0.0 a	0.7 a	0.0 a
	II	0.0 a	0.0 a	0.0 a	0.0 a
<i>F. fujikuroi</i>	I	2.2 ab	0.4 a	0.9 a	0.0 a
	II	0.2 a	0.0 a	0.2 a	0.0 a
Mean		9.3 b	5.0 a	3.6 b	1.4 a

Means followed by the same letter do not differ significantly at  $\alpha = 0.05$

The comparison of the species composition of isolates of the *Fusarium* genus showed that the *F. solani* fungi were found only in the epidermis of the spears (in both groups) collected from the older plantation. There were no *F. culmorum*, *F. equiseti*, and *F. proliferatum* found in the parenchyma of the spears without disease symptoms collected from the younger plantation. *F. fujikuroi* fungi were absent from the parenchyma of the spears collected from both the older and younger plantations (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*

The analysis of the white *A. officinalis* spears for the colonisation by fungi of the *Fusarium* genus depending on the age of the plantation revealed five fungal species colonising the plants in the first year of the research: *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, and *F. solani* (Tables 4 and 5).

*F. oxysporum* fungi were found in the spears of both groups. At the first and third harvest dates there were more *F. oxysporum* isolates obtained from the spears without disease symptoms collected from the older plantation. *F. proliferatum* fungi were found only in the spears collected at the second and third harvest dates, both in the group with and without disease symptoms. The count of fungi of the *Fusarium* genus increased at the subsequent harvest dates. In the second year of the research there were six species of fungi of the *Fusarium* genus isolated from the spears. *F. fujikuroi* was a new species, which was not isolated in the first year of the study. Both groups of spears (with and without disease symptoms) differed significantly in the counts of *F. oxysporum* and other species. *F. proliferatum* and *F. fujikuroi* were isolated only at the second and third harvest dates. The colonisation of the spears increased at the subsequent harvest dates. At the second and third harvest dates there were significant differences in the colonisation of the spears by all species of the *Fusarium* genus. This dependence was not observed in the group of spears without disease symptoms. Like in the previous years, in the third year of the study *F. oxysporum* isolates were the most numerous in both groups of spears. There were significant differences noted since the first harvest date. Like in the previous years, *F. proliferatum* and *F. fujikuroi* isolates were found only at the second and third harvest dates.

The comparison of the colonisation of the *A. officinalis* spears with disease symptoms by all fungal species of the *Fusarium* genus depending on the harvest date revealed significant differences between the first and subsequent harvest dates. These differences were not observed in the group of spears without disease symptoms (Table 4). There were statistically significant differences in the count of isolates of the *Fusarium* genus between the older and younger plantations only in the second year of the study (Table 4).

*The colonisation of white A. officinalis spears by fungi of the Fusarium genus depending on the age of plantation*

There was a significant correlation between the colonisation of the spears with disease symptoms by fungi of the *Fusarium* genus and the age of the plantation (the mean value for three years of the research). There were more isolates of the *Fusarium* genus found in the spears harvested from the older plantation (in the seventh, eighth, and ninth years of cultivation) than in those harvested from the younger plantation (in the fourth, fifth, and sixth years of cultivation). There were no significant differences in the count of isolates found in the spears without disease symptoms (Figure 3A).

In the subsequent years of observations, the spears with disease symptoms harvested from the older plantation were characterised by significantly higher fungal colonisation (Figure 3B–D).

This dependence was less marked in the group of spears without disease symptoms. There were statistically significant differences between the older and younger plantations in the count of isolates of the *Fusarium* genus only in the second year of the study (Figure 3C).

*Pathogenicity of the Fusarium genus*

Selected isolates of the *Fusarium* genus collected from different fragments of white spears differed in their pathogenicity against *A. officinalis* plants (Figure 4).

**Table 4.** The occurrence of various species the *Fusarium* genus in white *A. officinalis* 'Eposs' spears (with disease symptoms) at the consecutive harvest dates and years of the research (the mean value for the epidermis and parenchyma)

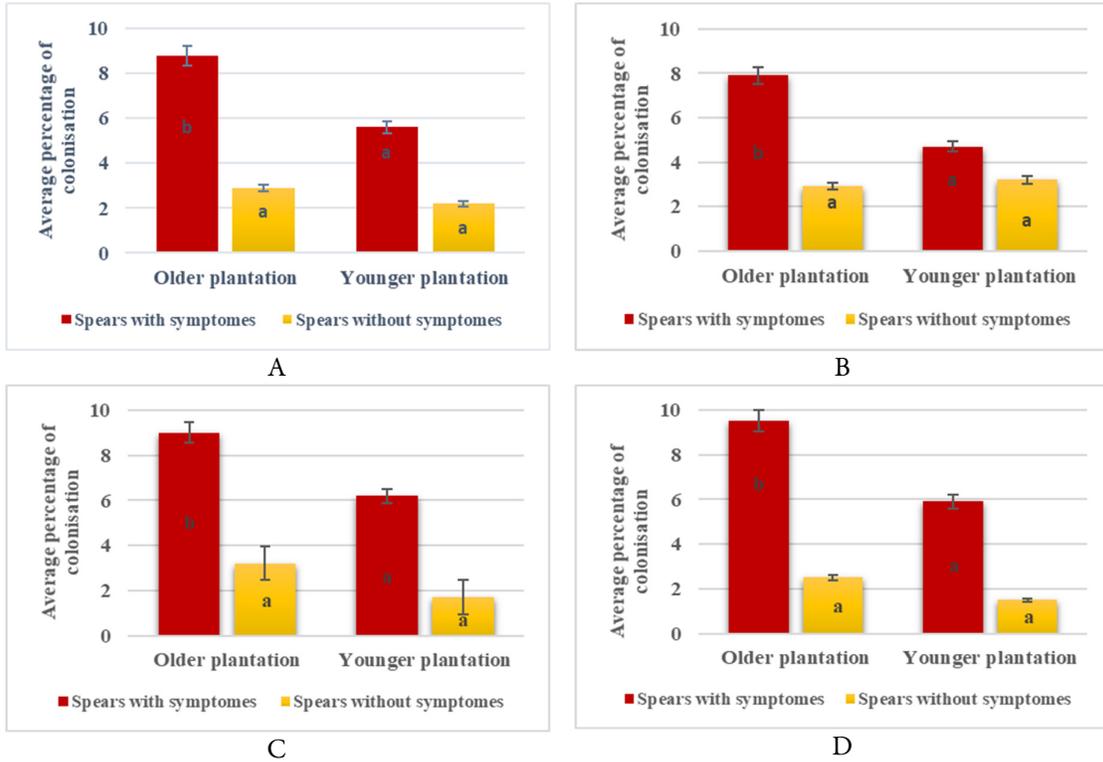
Species	Plantation	Frequency of isolates (%) in spears			
		1.	2.	3.	Mean
First year					
<i>F. culmorum</i>	I	5.0 a	8.0 ab	2.0 a	2.5 a
	II	0.0 a	0.0 a	0.0 a	
<i>F. equiseti</i>	I	7.0 a	5.0 a	6.0 a	4.0 a
	II	0.0 a	4.0 a	2.0 a	
<i>F. oxysporum</i>	I	17.0 bc	30.0 d	36.0 d	25.8 b
	II	17.0 bc	24.0 cd	31.0 d	
<i>F. proliferatum</i>	I	0.0 a	7.0 ab	10.0 ab	3.8 a
	II	0.0 a	4.0 a	2.0 a	
<i>F. solani</i>	I	3.0 a	2.0 a	4.0 a	1.5 a
	II	0.0 a	0.0 a	0.0 a	
Mean		4.9 a	8.4 ab	9.3 b	
Second year					
<i>F. culmorum</i>	I	2.0 ab	1.0 ab	0.0 a	1.0 a
	II	2.0 ab	1.0 ab	0.0 a	
<i>F. equiseti</i>	I	5.0 ab	5.0 ab	4.0 ab	3.0 a
	II	1.0 ab	2.0 ab	1.0 ab	
<i>F. oxysporum</i>	I	28.0 def	32.0 efg	39.0 g	30.2 c
	II	23.0 de	25.0 def	34.0 fg	
<i>F. proliferatum</i>	I	0.0 a	22.0 d	19.0 cd	10.3 b
	II	0.0 a	11.0 bc	10.0 abc	
<i>F. solani</i>	I	0.0 a	1.0 ab	1.0 ab	0.3 a
	II	0.0 a	0.0 a	0.0 a	
<i>F. fujikuroi</i>	I	0.0 a	2.0 ab	3.0 ab	1.0 a
	II	0.0 a	1.0 ab	0.0 a	
Mean		5.1 a	8.6 b	9.2 a	
Third year					
<i>F. culmorum</i>	I	3.0 ab	5.0 ab	2.0 ab	1.7 a
	II	0.0 a	0.0 a	0.0 a	
<i>F. equiseti</i>	I	4.0 ab	5.0 ab	6.0 ab	3.0 a
	II	1.0 ab	2.0 ab	0.0 a	
<i>F. oxysporum</i>	I	32.0 d	38.0 def	44.0 f	35.7 b
	II	23.0 c	42.0 ef	35.0 de	
<i>F. proliferatum</i>	I	0.0 a	9.0 b	9.0 b	3.5 a
	II	0.0 a	2.0 ab	1.0 ab	
<i>F. solani</i>	I	1.0 ab	3.0 ab	3.0 ab	1.2 a
	II	0.0 a	0.0 a	0.0 a	
<i>F. fujikuroi</i>	I	0.0 a	2.0 ab	5.0 ab	1.2 a
	II	0.0 a	0.0 a	0.0 a	
Mean		5.3 a	9.0 b	8.7 b	

Means followed by the same letter do not differ significantly at  $\alpha = 0.05$

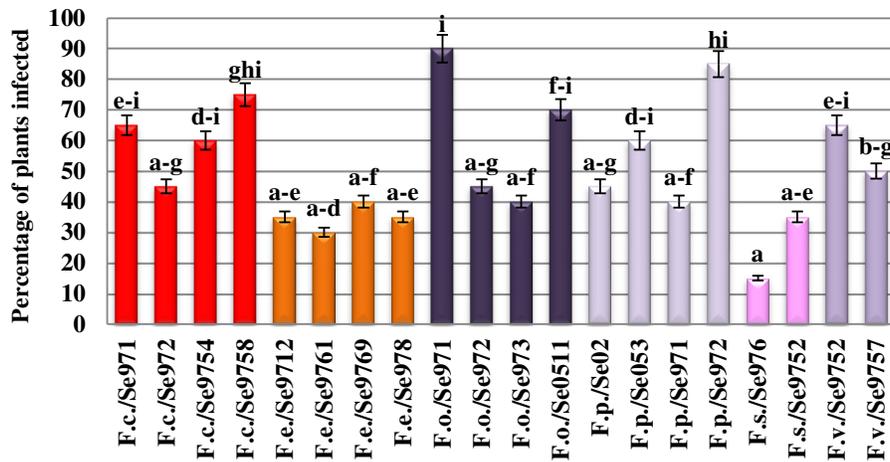
**Table 5.** The occurrence of various species the *Fusarium* genus in white *A. officinalis* 'Eposs' spears (without disease symptoms) at the consecutive harvest dates and years of the research (the mean value for the epidermis and parenchyma)

Species	Plantation	Frequency of isolates (%) in spears			
		1.	2.	3.	Mean
First year					
<i>F. culmorum</i>	I	3.0 a	1.0 a	0.0 a	1.0 a
	II	2.0 a	0.0 a	0.0 a	
<i>F. equiseti</i>	I	1.0 a	2.0 a	2.0 a	1.3 a
	II	0.0 a	1.0 a	2.0 a	
<i>F. oxysporum</i>	I	5.0 ab	10.0 b	24.0 c	15.3 b
	II	2.0 a	21.0 c	30.0 d	
<i>F. proliferatum</i>	I	0.0 a	3.0 a	2.0 a	0.8 a
	II	0.0 a	0.0 a	0.0 a	
<i>F. solani</i>	I	0.0 a	0.0 a	0.0 a	0.0 a
	II	0.0 a	0.0 a	0.0 a	
Mean		1.1 a	3.8 b	6.0 c	
Second year					
<i>F. culmorum</i>	I	3.0 ab	4.0 ab	0,0 a	1.3 a
	II	1.0 a	0.0 a	0,0 a	
<i>F. equiseti</i>	I	2.0 a	0.0 a	1,0 a	0.7 a
	II	0.0 a	1.0 a	0,0 a	
<i>F. oxysporum</i>	I	14.0 d	10.0 cd	15,0 d	10.8 b
	II	4.0 ab	8.0 bc	14,0 d	
<i>F. proliferatum</i>	I	0.0 a	3.0 ab	2,0 a	1.2 a
	II	0.0 a	0.0 a	2,0 a	
<i>F. solani</i>	I	0.0 a	1.0 a	1,0 a	0.3 a
	II	0.0 a	0.0 a	0,0 a	
<i>F. fujikuroi</i>	I	0.0 a	0.0 a	1,0 a	0.3 a
	II	0.0 a	0.0 a	1,0 a	
Mean		2.0 a	2.2 a	3.1 a	
Third year					
<i>F. culmorum</i>	I	2.0 abc	4.0 a-e	0.0 a	1.2 a
	II	0.0 a	1.0 ab	0.0 a	
<i>F. equiseti</i>	I	0.0 a	4.0 a-e	1.0 ab	1.3 a
	II	0.0 a	1.0 ab	2.0 abc	
<i>F. oxysporum</i>	I	10.0 e	9.0 de	8.0 cde	8.3 b
	II	7.0 b-e	8.0 cde	8.0 cde	
<i>F. proliferatum</i>	I	0.0 a	3.0 a-d	1.0 ab	0.8 b
	II	0.0 a	1.0 ab	0.0 a	
<i>F. solani</i>	I	0.0 a	1.0 ab	0.0 a	0.2 a
	II	0.0 a	0.0a	0.0 a	
<i>F. fujikuroi</i>	I	0.0 a	1.0 ab	2.0 abc	0.5 a
	II	0.0 a	0.0 a	0.0 a	
Mean		1.6 a	2.7 a	1.8 a	

Means followed by the same letter do not differ significantly at  $\alpha = 0.05$



**Figure 3.** The colonisation of the white *Asparagus officinalis* ‘Eposs’ spears by fungi of the *Fusarium* genus: A – the mean colonisation of the epidermis and parenchyma during the three years of the research, B – the mean colonisation of the epidermis and parenchyma in the first year of the research, C – the mean colonisation of the epidermis and parenchyma in the second year of the research, D – the mean colonisation of the epidermis and parenchyma in the third year of the research  
Means followed by the same letter do not differ significantly at  $\alpha = 0.05$



**Figure 4.** The pathogenicity of fungi of the *Fusarium* genus against *A. officinalis* plants in the growth chamber  
Means followed by the same letter do not differ significantly at  $\alpha = 0.05$

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./Se9758. There were also differences in the degree of infection of plants in the treatments where *F. oxysporum* isolates were used. The highest degree was observed in the treatments with the F.o./Se971 isolate collected from the epidermis of the spears with disease symptoms. In the treatment with the isolate, derived from the epidermis of white spears without disease symptoms, F.o./Se973 had the lowest plant infection. In treatments where the pathogenicity of *F. proliferatum* isolates was evaluated, the highest percentage of plants was infected by the F.p./Se972 isolate isolated from the parenchyma of spears without disease symptoms. There were no statistically significant differences in the pathogenicity in the treatments with *F. equiseti*, *F. solani*, and *F. fujikuroi* isolates. The tested isolates were characterised by different pathogenicity. Moreover, those with lower pathogenicity infected fewer plants.

## Discussion

The research showed that most of the isolates found in the white *A. officinalis* spears of the 'Eposs' cultivar belonged to the *Fusarium* genus. The following six species of this genus were identified: *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. fujikuroi*. Researchers all over the world focus on determining the economic significance of fusariosis on *A. officinalis*, the distribution of the disease in various regions of the world, and the assessment of factors causing the development of this disease. The occurrence of fusariosis is influenced by various factors, which are very difficult to determine precisely. Therefore, researchers determine the occurrence of various fungal species in geographical zones, isolate the most common of them in a particular region, determine the requirements necessary for their development, and assess the susceptibility of asparagus cultivars to fusariosis. Worldwide research has shown that fungi of the *Fusarium* genus colonising *A. officinalis* spears can be found in various geographical zones. Although they are cosmopolitan species, remaining in soil and on plants for a long time, there are significant differences in the structure of their populations, which are most likely to be affected by the specificity of a climate in a particular region. Among the numerous *Fusarium* species commonly occurring around the world *F. oxysporum*, *F. proliferatum*, and *F. culmorum* are the most frequently isolated. However, in some geographical regions less frequently isolated species may also be important, depending on external conditions. As results from scientific research, the most common fungi of the *Fusarium* genus found on *A. officinalis* in Europe were: *F. oxysporum* f. sp. *asparagi*, *F. redolens* f. sp. *asparagi*, and *F. culmorum* (Blok and Bollen, 1997, Blok *et al.*, 1997). The first two species were considered to be the cause of rhizome and root rot, whereas *F. oxysporum* was found to be the cause of wilting and stem base rot. Blok and Bollen (1997) and Blok *et al.* (1997) concluded that at the time of their study *F. proliferatum* was not a threat to the crops grown on the European plantations they analysed. Knaflewski and Sadowski (1990) and Weber and Knaflewski (1993) conducted their studies in Poland and arrived at a similar conclusion at that time. Our study revealed changes in the population of fungi of the *Fusarium* genus colonising *A. officinalis* spears. Although *F. oxysporum* and *F. culmorum* were the most frequently isolated species, other species were also identified. There were numerous *F. proliferatum* isolates on the white asparagus spears of the 'Eposs' cultivar. The change in the population of fungal species colonising *A. officinalis* spears may have been caused by recent climatic changes (Łykowski, 2007). Vujanović *et al.* (2006) draw attention to saprotrophic isolates of the genus *Fusarium*, which are able to colonise plant tissues, suggesting that perhaps such isolates are obtained from plants without clear disease symptoms. It cannot be excluded that among several hundreds of isolates that were isolated during the observations carried out in this study there were non-pathogenic isolates, however in infection tests it was shown that pathogenic isolates of all species were obtained from epidermis and parenchyma of spears, both from those with and without symptoms of disease. It was further shown that the isolates tested differed significantly in their pathogenicity. This variation was observed both between different species as well as within isolates of a single species.

The count of fungal isolates of the *Fusarium* genus found in the asparagus spears increased at the subsequent harvest dates. This means that the spears harvested at the latest date (in late June) were the most heavily infested with fungi. Vujanović *et al.* (2006) conducted an extensive study on the population of fungi of the *Fusarium* genus in Quebec, a province in eastern Canada. The authors selected 52 asparagus plantations located in several regions' representative of this province, which differed in the climate and soil conditions. Samples were collected in the last week of June, which is the last week of the harvest season in this area. The researchers chose that time because they believed that during that period asparagus had the lowest carbohydrate levels, which made the plants the most susceptible to fungal infestation. They analysed both healthy and infected plants and observed that the count of isolates of individual species may vary depending on the climatic conditions of the region. However, thanks to careful investigations, sometimes it is possible to isolate all species simultaneously from one plant, including plants without visible external symptoms of the disease. This fact was confirmed in our study, which showed that both the spears with and without disease symptoms were colonised by fungi of the *Fusarium* genus. However, there were always more isolates found in the spears with visible disease symptoms. Moreover, fungi of the *Fusarium* genus were found in the epidermis more frequently than in the parenchyma. According to Borrego-Benjumea *et al.* (2014), 93 *Fusarium* isolates obtained from plant and soil samples collected from commercial *A. officinalis* fields in southwestern Ontario were identified as *Fusarium oxysporum* (65.5%), *F. proliferatum* (18.3%), *F. solani* (6.4%), *F. acuminatum* (6.4%), and *F. redolens* (3.2%) on the basis of their morphological or cultural characteristics and analysis of the polymerase chain reaction (PCR) with species-specific primers. *In vitro* screening tests for pathogenicity showed that 50% of the field isolates were pathogenic to asparagus and 22% of the isolates caused the most severe disease symptoms in asparagus. According to Brizuela *et al.* (2020), the presence of fungi of the *Fusarium* genus is associated with Asparagus Decline Syndrome (ADS), which is a major phytosanitary problem on *A. officinalis* plantations worldwide. The researchers conducted their study on diseased plants and soil samples collected from 41 fields from the three major *A. officinalis* production regions in Spain. They identified eight *Fusarium* species in the soil: *F. oxysporum*, *F. proliferatum*, *F. redolens*, *F. solani sensu stricto*, *F. equiseti*, *F. culmorum*, *F. compactum*, and *F. acuminatum*. The most common species was *Fusarium oxysporum*. The authors proved that there was a statistical correlation between the *F. oxysporum* inoculum density and the average temperature in the warmest month. They also found a correlation between three cultivation factors (mean temperature, age of plantation, and *F. oxysporum* inoculum density) and the indicators of field diseases. They identified 13 *Fusarium* species on the roots of diseased plants, the most numerous of which were *F. oxysporum*, *F. proliferatum*, *F. oxysporum*, and *F. redolens*. These fungi were pathogenic to *A. officinalis* and were the main species associated with ADS. According to Farahanti-Kofoet *et al.* (2020), *F. oxysporum* f.sp. *asparagi*, *F. proliferatum*, and *F. redolens* are the species with the most negative influence on the growth and production of *A. officinalis*. Our study showed that the spears harvested at the latest date (late June) were the most heavily colonised by fungi. All the isolates of fungi of the *Fusarium* genus collected from the spears exhibited pathogenicity against *A. officinalis* plants. Elmer (2000) identified eight species of fungi of the *Fusarium* genus among 418 isolates collected from 1,776 *A. officinalis* spears. The researchers observed that *F. oxysporum* and *F. proliferatum* were the dominant species, with shares of 30% and 62%, respectively. The *Fusarium* colonisation was more intense in the basal segments (15.5%) than in the apical ones (8.1%). The researchers also observed that in both years of their study the frequency of occurrence of *F. proliferatum* in the spears purchased in June was higher than in those harvested in the other months. This finding is in line with the results of our study.

## Conclusions

Six species of fungi of the *Fusarium* genus were identified in the asparagus spears: *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. fujikuroi*. Among the *Fusarium* species colonizing *A.*

*officinalis* the greatest threat is *F. oxysporum*. Always there are more isolates in the spears with the symptoms, in epidermis. The late harvest date favors the development of fusariosis. This means that the spears harvested at the latest date (late June) are the most heavily colonised by fungi. The isolates of fungi of the *Fusarium* genus collected from the spears exhibit pathogenicity against *A. officinalis* plants.

### Authors' Contributions

Conceptualization: RA; methodology: RA; formal analysis RA, funding acquisition RA and BJ; writing-original draft RA, writing-review & editing RA and BJ. Both authors have read and agreed to the published version of the manuscript.

### Acknowledgements

The publication was co-financed within the framework of the Ministry of Science and Higher Education program as "Regional Initiative Excellence" in 2019-2022, Project No. 005/RID/2018/19, financing amount: 12 000 000 PLN.

### Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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