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PRELIMINARY RESEARCH ON PATHOGENIC FUNGI COLONIZING *ANTHOXANTHUM ARISTATUM* BOISS.

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Abstract

Pathogenic fungi were sampled in western Poland from cereals (*Triticosecale*, *Secale cereale* L.) and from the accompanying weed *Anthoxanthum aristatum* Boiss. – an invasive species for the flora of Poland, and Western and Central Europe. The most numerous pathogens were *Fusarium* spp., with 122 isolates obtained from *A. aristatum* and 98 from cereals, and *Alternaria* spp., with 124 isolates obtained from *A. aristatum* and 149 from cereals. In the tested cereals and weed, many other pathogen cultures were also detected (*Cladosporium* sp., *Epicoccum* sp., *Helminthosporium* sp., *Rhizoctonia* sp., *Stemphylium* sp., *Ulocladium* sp.). *A. aristatum*, as an invasive weed species, inhabited by a large number of pathogenic fungi, is a potential vector for fungal spread, especially in infertile crop fields, and also in anthropogenic habitats which *A. aristatum* has successfully colonised.

Key words: Anthoxanthum aristatum, Weeds, Cereals, Pathogenic fungi.

Introduction

Several monocotyledonous weeds accompany agricultural cultivation of cereals, oilseeds, and root crops. In Poland, *Apera spica-venti* (L.) P. Beauv., *Poa annua* L., *Bromus secalinus* L., *Avena fatua* L., *Echinochloa crus-galli* (L.) P. Beauv. and *Alopecurus myosuroides* Huds. have the greatest economic importance. *Anthoxanthum aristatum* Boiss. (annual vernalgrass) is one of a few species (apart from *A. fatua* L.), infesting cereal cultivations in oligotrophic habitats (Tokarska-Guzik, 2005). The natural range of the annual grass includes Atlantic-Mediterranean Europe and North-West Africa. Since the beginning of the 19th century, *A. aristatum* has spread successively in Western and then Central Europe, reaching the eastern borders of Poland, and has been considered as an invasive species (Tokarska-Guzik, 2005; Drapikowska *et al.*, 2013). The reasons for the efficient grass expansion are high fertility and the ability of the seeds to germinate even after many years. Furthermore, *A. aristatum* secretes into the soil metabolites harmful for seedlings of associated species, such as cyanogenic glucosides and coumarin (Latowski, 2005). In Poland, occurrence of the weed is limited to rye and triticale cultivated in oligotrophic habitats (Drapikowska *et al.*, 2020). In the areas mentioned above, organic farming is often carried out without the use of pesticides, providing grain for the production of "organic food" – groats, cookies, and baking bread.

Plant pathogenic fungi can be spread or survive by infected seeds and remains of infected plants. The source of the pathogen inoculum can also be infected weeds remaining in the field. It is widely known that lack of proper crop rotation combined with the abandonment of chemical protection may have consequences in the form of soil accumulation of the pathogens. Moreover, lack of removal of weeds, which are a pathogen reservoir, contributes to inefficiencies in crop protection against these pathogens (Wisler & Norris, 2005). Plant diseases are the result of interactions between the host organism, the pathogen, and the environment in which they are located. This interaction creates the "disease triangle."

A. aristatum is one of the plant species rarely affected by pathogenic fungi. The reason for this phenomenon is connected with the high content of allelopathic coumarin in tissues (Connor, 2012). So far, several species of pathogenic fungi have been described in *A. aristatum* in Europe, Australia, and New Zealand (Farr & Rossman, 2020). Among others, *A. aristatum* pathogens include the most important cereal pathogens – *Blumeria graminis* and *Puccinia graminis. B. graminis* causes powdery mildew – one of the most common and destructive diseases of cereals and grasses. *P. graminis* causes black rust, a significant disease of wheat, triticale, rye, oats, barley, and various wild grasses (Farr & Rossman, 2020). Other pathogens of *A. aristatum* include *Tilletia anthoxanthi* causing bunt disease also on *A. odoratum* and *A. alpinum*, and the species *Tilletia holci*, pathogenic for the genera *Anthoxanthum* and *Holcus* (Kokes, 2010; Farr & Rossman, 2020). Currently, there is little information regarding stem base disease occurring on *A. aristatum.* In cereal crops, species of the genera *Oculimacula*, *Rhizoctonia*, *Gaeumannomyces*, and *Fusarium* cause significant losses by limiting plant growth and yield. *Fusarium* species are also essential pathogens of cereals, causing seedling blight, foot rot, and head blight. Contamination of cereal grain by *Fusarium* mycotoxins (deoxynivalenol, nivalenol, and zearalenone) is particularly harmful to human and animal health (Doohan *et al.*, 2003). In green crops grown without the use of chemical protection, mycotoxin contamination of crops seems to be especially important. Recently, new pathogens of the *Fusarium* genus – *F. cerealis* and *F. avenaceum* – on stalks *A. aristatum* have been found (Pieczul *et al.*, 2018, 2019). The presence of *F. avenaceum* was described previously in the related species *Anthoxanthum odoratum* L. (Harrow *et al.*, 2010).

This study aimed to investigate the stem base infestation of *A. aristatum* Boiss. by pathogenic fungi, in particular species of the genus *Fusarium*. We also compared, in the same locations, the colonization of cereals by these pathogens, indicating *A. aristatum* as a source of pathogen infection.

Materials and Methods

Individuals of *A. aristatum* and crop cereal (rye, triticale) were collected in 30 populations growing in western Poland (Tables 1 and 2). The plants had no disease symptoms on the leaf; however, on the stem bases, many disease symptoms were noted and selected for detailed mycological analysis. Isolation of fungal cultures was done immediately after collecting the plant material. From each location, 20 randomly selected *A. aristatum* stems and 20 fragments of the stalk of the cereal stem in which weed occurred were collected for examination. Small pieces of stalks (3-5 mm) were sterilized for 40 s in a solution containing: <5% sodium carbonate, <1% sodium hydroxide, <5% sodium hypochlorite (ACE, Procter and Gamble bleach), rinsed twice in sterile distilled water, then they were dried and put on PDA (Potato Dextrose Agar) medium. Fungal colonies were transplanted into a new PDA medium. *Fusarium* spp. cultures to provoke sporulation were additionally allocated to selective nutrient agar (SNA) (Nelson *et al*., 1983). The culture was carried out at 21°C. After seven days of incubation, individual cultures were subjected to mycological analysis. The morphological identification of fungi of the genus *Fusarium* was confirmed based on TEF (translation elongation factor) and ITS (internal transcribed spacer) sequence analysis, selected isolates representing the identified species with data contained in the GenBank database (https://www.ncbi.nlm.nih.gov).

Results

Over 700 fungal cultures were obtained in the studies. Three hundred forty-three isolates from *A. aristatum* and 314 from cereals have been assigned to the species or genus level. The most numerous genera were *Fusarium* spp. (122 isolates obtained from *A. aristatum* and 98 from cereals) and *Alternaria* spp. (124 isolates obtained from *A. aristatum* and 149 from cereals). Strains of the genera *Alternaria* and *Fusarium* were also the most common, occurring in most of the studied locations. Additionally, on the examined plant material, many other cultures were obtained: *Cladosporium* sp., *Epicoccum* sp., *Helminthosporium* sp., *Rhizoctonia* sp., *Stemphylium* sp., *Ulocladium* sp. Detailed data on abundance and location are shown in Table 1. All *Fusarium* isolates were identified to the species level. In total, seven species were identified: *F. avenaceum*, *F. cerealis*, *F. culmorum*, *F. equiseti*, *F. oxysporum*, *F. sporotrichioides*, and *F. tricinctum*. The most numerous were *F. culmorum* (38 isolates from *A. aristatum* and 30 from cereals), *F. avenaceum* (23 isolates from *A. aristatum* and 18 from cereals), and *F. oxysporum* (27 isolates from *A. aristatum* and 27 from cereals). Details of the isolation related to individual locations are presented in (Tables 1 and 2).

Discussion

Many plant pathogens are not necessarily closely associated with a single host species but attack many different cultivated species, as well as weeds accompanying these cultivations. The weeds mediate in the transmission of diseases and pests of crops and cause much higher yield losses than diseases or pests only. They form a bridge allowing pathogenic fungi to survive the winter period. Infected seeds are a grave source of primary infection for crop plants. The existing research shows that grass seeds are predominantly colonized by fungi (Kiecana *et al*., 2012).

Various species of monocotyledonous weed have been reported to date as hosts for fungi pathogenic for cereals. Among others, the hosts for *B. graminis* include *A. spica-venti*, *A. fatua*, *Elymus repens* L., and *B. secalinus*; *Puccinia graminis*, *Puccinia striiformis* and *Puccinia recondita* were described as pathogenic for *A. spica-venti*, *A. fatua*, *E. repens*, *B. secalinus*, *E. crus-galli* and *P. annua* (Farr & Rosmann, 2020). The importance of *A. aristatum* in the expansion of pathogenic fungi onto cultivated cereals has not been studied satisfactorily so far. Notably, there is little information about the colonization of weeds by fungi, causing root and stem base diseases of cereals, and their importance in the formation of the inoculum reservoir. A single report indicated *B. graminis* as a pathogenic species for *A. aristatum*. During the conducted analysis, we did not find any disease symptoms on leaves of the tested weed. At the same time, several symptoms were observed on the stalks of weed and cereal plants, and they became the object of the study. It should be mentioned that most of the described species have been identified both on *A. aristatum* and on cereals. It indicates that isolated species of fungi are capable of infesting crops and accompanying weeds. Conducted mycological investigations result in many isolates of *Alternaria*, *Cladosporium*, *Epicoccum*, *Stemphylium*, *Ulocladium* obtained. In the cereal crops, most of these species are considered as saprotrophs colonizing dead plant tissues. These ubiquitous species are found on all parts of plants, and rarely pose a real threat to plant health, excluding black point – the cereal grain disease. Only under favourable conditions can they cause weakness or diseases of plants. However, we have been able to identify strictly pathogenic species. To our knowledge, we are the first to describe the occurrence of *Rhizoctonia* sp. on *A. aristatum.* This species in cereal crops causes sharp eyespot – a common disease of the stem base of cereals. The most exciting observation was the simultaneous identification of several species from the *Fusarium* genus on the tested weed and cereals.

Among the fungi inhabiting *A. aristatum* stalks, *Fusarium* species are the most important. Due to produced mycotoxins, their presence in cereal crops is potentially dangerous to human and animal health (Ferrigo *et al*., 2016). It should be mentioned that *Fusarium* mycotoxins can migrate in the agricultural environment, from the field through the soil and ending in surface and groundwater. Additionally, *Fusarium* can use both ammonium and nitrate forms of nitrogen in the soil, competing with both antagonistic fungi and plants (Celar, 2003). Because *A. aristatum* is a weed associated with crops in poor soils, this pathogen is an element of competition for such relatively weak environmental resources. In particular, bearing in mind the fact that *A. aristatum* is characterized by a short vegetation cycle and pathogens colonise kernels and harvest residues, it constitutes a source of infection in consecutive years. There are known connections between primary *Fusarium* inoculum on gramineous weeds, crop residues, and soil samples and the final contamination of wheat in the fields (Landschoot *et al*., 2011). In this study, except *Rhizoctonia* sp. and *Fusarium* spp., we did not identify other pathogens of a complex of cereal root and stem base diseases, such as *Oculimacula yallundae* and *Oculimacula acuformis* (eyespot), *Gaeumannomyces graminis* (take all), *Drechslera sorokiniana* (foot rot), or *Typhula incarnata* (speckled snow rot) (Crous *et al*., 2003).

Table 1. Number of isolates of other fungi obtained from *Anthoxanthum aristatum* and the cereal stem base.

L.p.	Location		*Alternaria* spp.		*Fusarium* spp.		*Cladosporium* sp.		*Epicoccum* sp.		*Rhizoctonia* sp.		*Stemphylium* sp.		*Ulocladium* sp.		Others	
	N	E	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C
1.	52,093502	16,033987	3	2	3	1											6	4
2.	52,084217	16,032879	2	3	13	9	1		2		2						1	1
3.	52,079945	16,035477	2	4	2	4			2								7	3
4.	52,10771	16,010218	5	7	5	4		2	2	2			1				3	2
5.	52,108196	16,00902	4	4	1	4		2	3	1							8	4
6.	52,10991	16,014174	3	6	6	2			1		4	1					3	5
7.	52,12137	16,010849	5	4	5	3		1		1							3	4
8.	52,11318	16,002283	3	9	3	1			2								2	8
9.	52,106026	15,936371	2	3	2	5			4		2						3	4
10.	52,11188	15,93807	3	12														8
11.	52,139187	15,950378	2	6	6	7			1	1					1		4	3
12.	52,142216	15,950261	10	7				1	4	2							6	5
13.	52,14557	15,9511364	2	4	2	2			6		2	3			1		5	3
14.	52,15036	15,944679	3	2	2				2	1	2	3					7	5
15.	52,131245	16,020935	9	5					2				1				6	7
16.	52,130054	16,008512	4	2	6	10	1	1	1						1		7	5
17.	52,140114	16,06425	8	4	2	3			3	2							3	5
18.	52,137554	16,067932	5	6	4	3			3								5	6
19.	52,130527	16,074203	3	8				2	7								4	2
20.	52,133553	16,06343	5	5	3	1			2								6	4
21.	52,153473	16,069708	4	3	9	8				11	3						3	2
22.	52,17264	16,065712	4	8			1	3	2	2	2	1					7	5
23.	52,1696	16,06718	3	7	7	2		2	3	2							5	4
24.	52,199364	16,05514	6	5	3				2								6	6
25.	52,19255	16,002222	3	5	2	1			2								9	9
26.	52,20743	15,984972	3	4	3	6			3	1			2				5	7
27.	52,209625	15,993323	3	5	10	4											3	6
28.	52,21857	15,976498	7	3		2		1	3	2						1	2	3
29.	52,219223	15,981906	4	4	5	5			4	2							2	3
30.	52,223682	15,980329	4	2	8	11		1							3	1	5	3

Abbreviations: A – Anthoxanthum, C- cereal

Table 2. Number of fungal isolates from the genus *Fusarium* obtained from *Anthoxanthum aristatum* and the cereal stem base.

L.p.	Location		*F. avenaceum*		*F. acuminatum*		*F. cerealis*		*F. culmorum*		*F. equiseti*		*F. oxysporum*		*F. sporotrichioides*		*F. tricinctum*	
	N	E	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C
1.	52,093502	16,033987	3	1														
2.	52,084217	16,032879	1	1	4	3	2		4	2	1	2				1	1	
3.	52,079945	16,035477							2	3				1				
4.	52,10771	16,010218	1	2					2	1			2	1				
5.	52,108196	16,00902	1	2		2												
6.	52,10991	16,014174							4	2	2							
7.	52,12137	16,010849											5	3				
8.	52,11318	16,002283						1	3									
9.	52,106026	15,936371								2			2	3				
10.	52,11188	15,93807																
11.	52,139187	15,950378	2	2											4	5		
12.	52,142216	15,950261																
13.	52,14557	15,9511364											2	2				
14.	52,15036	15,944679							2									
15.	52,131245	16,020935																
16.	52,130054	16,008512							3	10			3					
17.	52,140114	16,06425	2	3														
18.	52,137554	16,067932	4	3														
19.	52,130527	16,074203																
20.	52,133553	16,06343	3	1														
21.	52,153473	16,069708	1	2			1	3	7	3								
22.	52,17264	16,065712																
23.	52,1696	16,06718							4	1			2		1	1		
24.	52,199364	16,05514	2								1							
25.	52,19255	16,002222									2	1						
26.	52,20743	15,984972		1									3	3		2		
27.	52,209625	15,993323	1		4		1		4	4								
28.	52,21857	15,976498														2		
29.	52,219223	15,981906	2										3	5				
30.	52,223682	15,980329							3	2			5	9				

Abbreviations: A – Anthoxanthum, C- cereal

The results of our research have shown that, despite the content of coumarin, *A. aristatum* is a host of many pathogens not described before, which simultaneously inhabit cereals. The presented investigations confirmed that this grass constitutes a reservoir of numerous fungal pathogens: members of *Fusarium* and *Rhizoctonia*, among which there are taxa described by the authors of this paper for the first time. The mass occurrence of *A. aristatum* has been observed in many sites in Central Europe. Their number has repeatedly affected the structure and floristic composition of communities. The seed bank of *A. aristatum* is very abundant (Drapikowska, 2013), and there is a risk of proliferation of fungal pathogens even after many years. It follows that the disease can be managed using three strategies: exclusion or reduction of the pathogen inoculum, an increase of host resistance or modification of the environment, as well as the elimination of vector organisms so that the development of infection is not possible. In some situations, it is possible to modify the environment so that it is optimal for host growth and not suitable for epidemic development. Liming the soil as a pH-increasing treatment results in the rapid withdrawal of *A. aristatum* in the field (authors' observation).

In conclusion, the increasing frequency of weed infestation is the effect of irrational crop rotation, in which 75% of crops are cereals. Particular emphasis should be placed on combating fungal pathogens but also on combating weeds that have colonized cereal crops in poor habitats over several decades, becoming a reservoir of fungal diseases in these fields. It should be noted that the remains of infected plants are a source of infection in the following years, and weeds in the field of crops, depending on the species represented, have a variety of harmful effects.

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