

## Article

# New Pathotype Nomenclature for Better Characterisation the Virulence and Diversity of *Blumeria graminis* f.sp. *avenae* Populations

Sylwia Okoń <sup>1</sup>, Magdalena Cieplak <sup>1</sup>, Adam Kuzdraliński <sup>2</sup> and Tomasz Ociepa <sup>1,\*</sup>

<sup>1</sup> Institute of Plant Genetics, Breeding and Biotechnology, University of Life Science in Lublin, Akademicka 15, 20-950 Lublin, Poland; sylwia.okon@up.lublin.pl (S.O.); magdalena.cieplak@up.lublin.pl (M.C.)

<sup>2</sup> Department of Biotechnology, Microbiology and Human Nutrition, University of Life Sciences in Lublin, Skromna 8, 20-704 Lublin, Poland; adadamkuzdralinski@gmail.com

\* Correspondence: tomasz.ociepa@up.lublin.pl

**Abstract:** Fungal cereal pathogens, including *Blumeria graminis* f.sp. *avenae*, have the ability to adapt to specific conditions, which in turn leads to overcoming host resistance. An important aspect is the standardized way of characterizing the races and pathotypes of the pathogen. In the presented work, for the first time it was proposed to use a unified letter code that allows describing the pathotypes of *B. graminis* f.sp. *avenae*. The set of 14 oat genotypes were used as a differential set. This set included genotypes having so far described powdery mildew resistance genes *Pm1–Pm11*, and two genotypes (*A. sterilis* and *A. strigosa*) with effective sources of resistance to *Bga*. Based on the analysis of 160 *Bga* isolates collected in 2016–2019 from 4 locations in Poland, the most numerous was the TBBB pathotype, represented by 30% of the tested isolates. It was present in all analyzed populations. Subsequently, 8.1% and 6.3% of the isolates represented the TBCB and RBBB pathotypes, respectively.

**Keywords:** *Avena*; diversity; oat; pathotype; powdery mildew



**Citation:** Okoń, S.; Cieplak, M.; Kuzdraliński, A.; Ociepa, T. New Pathotype Nomenclature for Better Characterisation the Virulence and Diversity of *Blumeria graminis* f.sp. *avenae* Populations. *Agronomy* **2021**, *11*, 1852. <https://doi.org/10.3390/agronomy11091852>

Academic Editors: Diego Rubiales and Jerzy Henryk Czembor

Received: 8 August 2021

Accepted: 13 September 2021

Published: 15 September 2021

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

Plant diseases are the result of a complex interaction between a sensitive host, a virulent pathogen, and favorable environmental conditions [1,2]. *Blumeria graminis* is a pathogen that spreads mostly by anamorphic conidia, but survives unfavorable conditions through a telomorphic stage, which terminates by the production of chasmothecia with numerous asci containing ascospores [3]. Climate change may contribute to conditions for better pathogen survival, as well as an increase in pathogenicity and faster spread [4,5].

*Blumeria graminis* f.sp. *avenae* (*Bga*) is one of the most dangerous oat fungal pathogens [6]. It is common in central and north-western Europe and North America [7,8]. The disease is also a serious threat in Eastern European countries [9]. In addition, in recent years, the disease has spread to areas where its symptoms had not previously been observed. Literature sources report the emergence of the disease, for example, in China [10] or the north-western Himalayan region [11].

Due to the increasing spread of the pathogen, its adaptability, and ability to evolve and overcome host resistance, continual research on monitoring of virulence are necessary. This research is also very important in order to prevent large-scale epidemics [12]. Conducting this type of research will allow for better planning of the strategy of plant protection against pathogens attacking. Therefore, the first goal of the presented study was to determine the virulence and diversity of *B. graminis* f.sp. *avenae* populations occurring in Poland in 2016–2019. These studies are a continuation of the observations started in 2010.

In pathogenicity monitoring studies, very important is the ability to compare results obtained in different regions of the world. An important aspect is the standardized way of characterizing the races and pathotypes of the pathogen. In the presented work, we would

like to propose the use of a unified letter code allowing describing *B. graminis* f.sp. *avenae* pathotypes, based on the description of other fungal pathogens affecting cereals [13–15]. The use of the standardized characteristics of pathotypes in further work on *B. graminis* f.sp. *avenae* will allow a reliable comparison of the results of the research carried out in various research centres. It will also allow monitoring of the diversity of the *B. graminis* f.sp. *avenae* population and the speed of changes taking place in the population in different regions of the world.

## 2. Materials and Methods

### 2.1. Location of Pathogen Populations and Dates of Sampling

The pathogen samples were collected for four years from 2016 to 2019 in four the same locations in Poland (Figure 1). Each separate population consisted of isolates collected in one year, with the total number of 40 (10 isolates from each location). Leaves of oat cultivars (*Avena sativa* L.) infected with *B. graminis* f.sp. *avenae* were originally collected randomly from fields belonging to plant breeding companies.



**Figure 1.** Geographic distribution of the locations from which the *Bga* isolates were collected.

### 2.2. Multiplication of Inoculum

Samples of *B. graminis* f.sp. *avenae* were obtained from infected leaves of random cultivars collected from each location. The distance between the sampling sites within a field was at least 5 m. Under laboratory conditions, from every sample, single spore isolates were obtained in accordance with the methodology previously described by Hsam et al. [16].

### 2.3. Differential Sets and Inoculation of the Leaf Segments

The set of 14 oat genotypes were used as a differential set. This set included genotypes having so far described powdery mildew resistance genes *Pm1–Pm11*, and genotypes *A. sterilis* [17] and *A. strigosa* [18] with effective sources of resistance to *B. graminis* f.sp. *avenae*. The control set also included the Fuchs cultivar susceptible to powdery mildew infection (Table 1). Seeds of each differential were sown in a pot filled with gardening peat substrate and placed in a mildew-proof growing chamber under natural daylight.

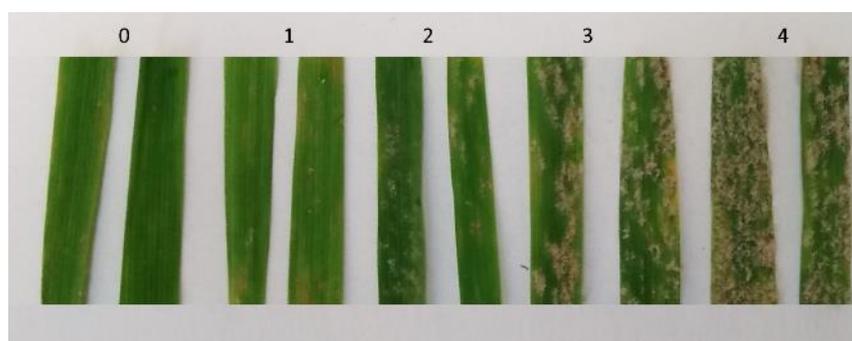
**Table 1.** Standard differential set of oat lines and cultivars with known resistance genes used to characterize virulence structure of the *Blumeria graminis* populations on oat in Poland across 2016–2019 [16,18–21].

Cultivar/Line	Gene Symbol	Host Set	Pedigree
Jumbo	<i>Pm1</i>	1	Flämingsstern/AJ20–61//Faggot
CC3678	<i>Pm2</i>	2	<i>A. hirtula</i>
Mostyn	<i>Pm3</i>	1	05443/Condor
Av1860	<i>Pm4</i>	2	<i>A. sativa</i> / <i>A. barbata</i>
Am27	<i>Pm5</i>	2	<i>A. sativa</i> / <i>A. macrostachya</i>
Bruno	<i>Pm6</i>	1	Halla/Gambo
APR122	<i>Pm7</i>	2	<i>A. sativa</i> / <i>A. eriantha</i>
Canyon	<i>Pm7</i>	3	<i>A. sativa</i> / <i>A. barbata</i>
Rollo	<i>Pm3 + Pm8</i>	1	LP75-512/W17286
AVE2406	<i>Pm9</i>	3	<i>A. byzantina</i>
AVE2925	<i>Pm10</i>	3	<i>A. byzantina</i>
CN113536	<i>Pm11</i>	3	<i>A. sativa</i> / <i>A. sterilis</i>
CN67383	<i>U<sub>A.ster.</sub></i>	4	<i>A. sterilis</i>
Pl 51586	<i>U<sub>A.stri.</sub></i>	4	<i>A. strigosa</i>
Fuchs	-	-	-

Three leaf segments of each differential were placed in 12-well culture plates with 6 g/L agar and 35 mg/L benzimidazole. The plates with the leaf segments were inoculated in a settling tower by spreading 500–700 powdery mildew spores per 1 cm<sup>2</sup>. The plates were then incubated in a growing chamber at 17 °C and an illuminance of approximately 4 kLx.

#### 2.4. Virulence Determination, Pathotype Designation, and Distribution

The reaction type of each differential was determined 10 days after inoculation and scored according to a 0–4 modified scale [22]; where 0 = no infection, no visible symptoms; 1 = highly resistant, fungal development limited, no sporulation; 2 = moderately resistant, moderate mycelium with some sporulation; 3 = moderately susceptible, extensive mycelium, more sporulation; 4 = highly susceptible, large colonies, and abundant sporulation (Figure 2). If disease symptoms were scored as 0, 1, or 2, the isolates were classified as avirulent to known genes against oat powdery mildew. If disease symptoms were scored as 3 or 4, the isolates were classified as virulent.



**Figure 2.** Photo of leaf fragments showing the different types of plant response to Bga infection.

The compiled reaction type data for each isolate to differential genotypes were coded as individual pathotypes (Table 2). In order to standardize the *B.graminis* f.sp. *avenae* isolates nomenclature, we propose to use a new letter code adapted from the available systems of the nomenclature of *P.graminis* f.sp. *tritici* [14], *P.recondita* f.sp. *tritici* [15] *P.coronata* f.sp. *avenae* [13].

**Table 2.** Code of 14 differentials for identification of *Blumeria graminis* f.sp. *avenae* pathotypes.

Letter Code.	Host Set 1	<i>Pm1</i>	<i>Pm3</i>	<i>Pm6</i>	<i>Pm8</i>
	Host Set 2	<i>Pm2</i>	<i>Pm4</i>	<i>Pm5</i>	<i>Pm7a</i>
	Host Set 3	<i>Pm7b</i>	<i>Pm9</i>	<i>Pm10</i>	<i>Pm11</i>
	Host Set 4	<i>A.ster</i>	<i>A.st</i>	-	-
B		L	L	L	L
C		L	L	L	H
D		L	L	H	L
F		L	L	H	H
G		L	H	L	L
H		L	H	L	H
J		L	H	H	L
K		L	H	H	H
L		H	L	L	L
M		H	L	L	H
N		H	L	H	L
P		H	L	H	H
Q		H	H	L	L
R		H	H	L	H
S		H	H	H	L
T		H	H	H	H

In the proposed isolate nomenclature system, the level of infection of the set of control genotypes was divided into two classes: low (L) and high (H). Low levels of infection were reported as 0, 1, or 2 and classified the plants as resistant and the isolates as avirulent. The high level of infection was described as 3 and 4 and classified the plants as susceptible and the isolates as virulent.

### 2.5. Data Analysis

Parameters for comparing all *B.graminis* f.sp. *avenae* populations were calculated on the basis of isolate virulence patterns on the set of differential genotypes. Virulence frequency ( $p$ ) as  $p = x/n$  (where  $x$  is the number of times a virulent reaction type was detected, and  $n$  is the total number of samples tested in a particular year) was calculated for each year. The total number of virulent reaction types for each isolate was calculated and reported as the virulence complexity. The frequency of the virulence complexity was determined for each year. Diversity within populations and pairwise distance between populations were assessed using different types of parameters: genetic diversity like Simpson ( $S_i$ ) and Shannon ( $S_h$ ) and genetic distance (Rogers index- $R$ ) based on the pathotype structure of populations; gene diversity like Nei index ( $H_s$ ) which is equivalent to a measure of the average dissimilarity within a population ( $ADW_m$ ) regarding the simple mismatch coefficient  $m$ , and the Nei gene distance ( $N$ ) based on the population virulence, and genetic diversity ( $KW_m$ ) and distance ( $KB_m$ ) measured by the Kosman indices, based the population pathotype and virulence structure [23–25]. All computations of populations parameters were performed with the HaGiS program [26] and the VAT software [25,27].

## 3. Results

### 3.1. Virulence Frequency

*B. graminis* f.sp. *avenae* isolates belonging to the analyzed populations collected in 2016–2019 showed a high level of virulence in relation to the control forms containing the *Pm1*, *Pm3*, *Pm6*, and *Pm3 + 8* genes. The average value of the virulence frequency of all analyzed isolates to these genes was 92.5%, 85.6%, 87.5%, and 85.6%, respectively. A low level of virulence was observed for the control forms with the *Pm9*, *Pm10*, and *Pm11* genes. In each of the analyzed populations, virulent isolates for these genes were identified, but their number was relatively small, and the low frequency of virulence allows these

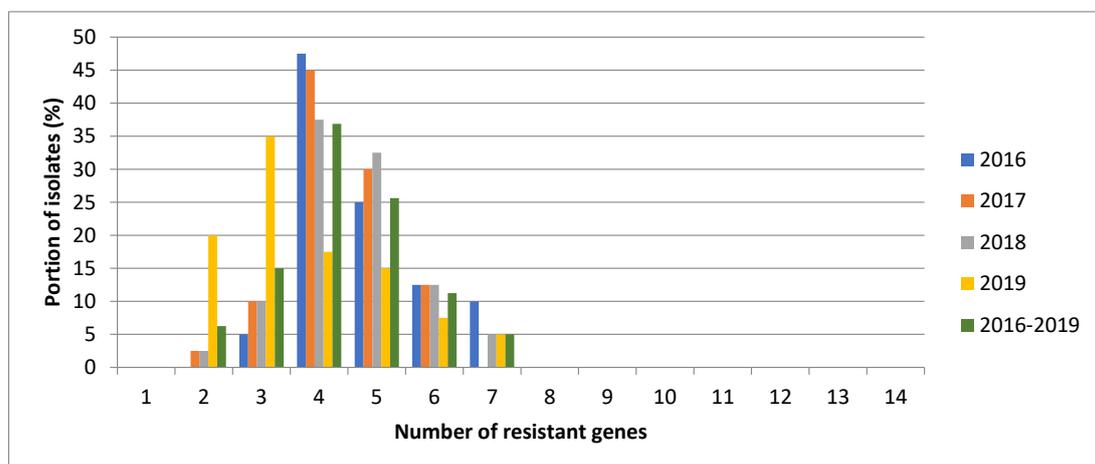
genes to be considered effective. Among the analyzed isolates, several virulent to the *Pm2* gene (8 from the 2019 population) and *Pm7* from the Canyon cultivar (5 from the 2016 population) were identified. The control set also included the *A. strigosa* genotypes and one *A. sterilis* genotype, which showed high efficiency and are a valuable source of resistance to powdery mildew. Among the tested isolates, 6.3% and 17.5%, respectively, were virulent to these genotypes.

All tested isolates from four populations collected over four consecutive years were avirulent to the control forms containing the *Pm4*, *Pm5*, and *Pm7* genes (line APR122). Detailed results of the analysis of the virulence frequency of particular populations are presented in Table 3.

**Table 3.** Virulence frequencies of *Blumeria graminis* f. sp. *avenae* isolates sampled from oat in 2016–2019.

Cultivar	Gene	Frequency (%)				
		2016	2017	2018	2019	2016–2019
Jumbo	<i>Pm1</i>	100	77.5	97.5	95	92.5
CC3678	<i>Pm2</i>	0	0	0	20	5
Mostyn	<i>Pm3</i>	90	97.5	92.5	62.5	85.6
Av1860	<i>Pm4</i>	0	0	0	0	0
Am27	<i>Pm5</i>	0	0	0	0	0
Bruno	<i>Pm6</i>	100	100	87.5	62.5	87.5
APR122	<i>Pm7</i>	0	0	0	0	0
Canyon	<i>Pm7</i>	12.5	0	0	0	3.1
Rollo	<i>Pm3 + Pm8</i>	90	100	92.5	60	85.6
AVE2406	<i>Pm9</i>	7.5	10	30	7.5	13.8
AVE2925	<i>Pm10</i>	22.5	17.5	15	12.5	16.9
CN113536	<i>Pm11</i>	27.5	27.5	17.5	15	21.9
CN67383	<i>U<sub>A.ster.</sub></i>	20	10	22.5	17.5	17.5
PI 51586	<i>U<sub>A.stri.</sub></i>	5	0	2.5	17.5	6.3

Analyzing the complexity of the tested *B. graminis* f.sp. *avenae* isolates can be observed that these isolates most often overcame the resistance of 4 out of 14 genes included in the control set (37% of isolates), this relationship was present in each of the analyzed populations. A total of 26% of the isolates overcame the resistance of 5 genes simultaneously, 15% overcame the resistance of 3 genes, and 11% of 6 genes simultaneously. The negligible number of isolates of 6 and 5% broke the resistance of 2 and 7 genes simultaneously. None of the tested isolates were able to break the resistance of 1, 8, 9, 10, 11, 12, 13, or 14 genes (Figure 3).



**Figure 3.** Virulence frequency of the analyzed *Bga* populations in particular years.

### 3.2. New Nomenclature for *B. graminis* f.sp. *avenae* Phenotypes

To create the *B. graminis* f.sp. *avenae* isolate nomenclature system, all the cultivars and lines with the powdery mildew resistance genes and 2 additional genotypes identified in our previous research as effective against powdery mildew, were used (Table 1). In the proposed system, the reference lines were divided into four groups depending on their reaction to *B. graminis* f.sp. *avenae* isolates. Information on the characteristics of control genotypes was collected on the basis of the available literature and ongoing own observations.

The first subgroup included Jumbo with the *Pm1*, Mostyn with *Pm3* and Bruno with *Pm6* genes. These genes have been present in many cultivars for many years [16,19,28–30]. Due to the long-term presence of these genes in cultivated forms, their level of resistance is currently very low. Most of the *B. graminis* f.sp. *avenae* isolates tested so far have broken the resistance of these genes; however, single isolates avirulent towards these genes are identified [31]. This subgroup also includes the *Pm8* gene, which was identified in the Rollo cultivar together with the *Pm3* gene [32]. Due to the lack of a line with a single *Pm8* gene, this cultivar was included in the control set. Numerous of our own observations show that the *Pm8* gene does not show a high level of resistance [33].

The lines with the *Pm2*, *Pm4*, *Pm5*, and *Pm7* genes form the second subgroup. These genes show a high level of resistance, probably due to the fact that they have not been widely used in oat breeding programs so far [16,19,20,29]. Introducing them to cultivated forms may induce the emergence of new pathogen pathotypes that will begin to break their resistance.

The third subgroup consist of genotypes with *Pm9* and *Pm10* genes, described by Herrmann et al. [20], showing a high level of resistance in the adult plant stage. Tests carried out at the seedling stage showed a high and moderate level of resistance of these genes (own observations). Lines with the *Pm11* gene identified by Ociepa et al. [34] showed a high and moderate level of resistance in the adult and seedling stage [21]. We also included the Canyon cultivar with the *Pm7* gene in this group. Numerous observations and tests conducted in recent years have shown that Canyon has a pattern of infestation different from the APR122 line. Due to the different reaction of these genotypes, we included both of them in the control set; for the sake of distinction, we marked them as *Pm7a* for the APR 122 line and *Pm7b* for Canyon.

In the fourth group, we placed two genotypes, *A. sterilis* and *A. strigosa*, identified in our previous work as effective sources of resistance to powdery mildew [17,18].

For each group, 16 combinations of high or low infection are possible. Each combination has an assigned letter characterizing a given group of genotypes. As a result, a 4-letter code will be used to describe the virulence of the *B. graminis* f.sp. *avenae* isolate. For example, an isolate marked as TBBB shows a high infection level in relation to the genotypes placed in the first group—it is virulent toward them and breaks the resistance of the *Pm1*, *Pm3*, *Pm6*, and *Pm8* genes. This isolate is avirulent toward genotypes from groups 2, 3, and 4 and does not break the resistance of the *Pm2*, *Pm4*, *Pm5*, *Pm7a*, *Pm7b*, *Pm9*, *Pm10*, and *Pm11* genes and the resistance sources identified in *A. sterilis* and *A. strigosa*.

The division into low and high virulence levels is very conventional. Readings 0, 1, and 2 are classified as avirulent and readings 3 and 4 are classified as virulent. However, a reading of 2 indicates that the gene's resistance is starting to decline and further pressure could lead to a rapid breakdown of the resistance. In some studies, such as the effectiveness of resistance genes, it is important to identify isolates that begin to break down resistance to a small extent. Therefore, in our code, we suggest marking the readings of 2 as L (low infection) with the + sign at the end of the code and the gene symbols for which the readings were classified as 2, if it is required by the conducted analyses. For example, an isolate marked as TBBB + *Pm9* shows a high level of infection in relation to the genotypes placed in the first group—it is virulent toward them and breaks the resistance of the *Pm1*, *Pm3*, *Pm6*, and *Pm8* genes. This isolate is avirulent towards genotypes from groups 2, 3, and 4 and does not break the resistance of the *Pm2*, *Pm4*, *Pm5*, *Pm7a*, *Pm7b*, *Pm9*, *Pm10*, and *Pm11*, *A. sterilis* and *A. strigosa* genes, but in the case of the *Pm9* line its reaction was

marked as 2. This may indicate that the level of virulence for this gene is increasing and isolates may arise that will completely break its resistance. This representation of virulence will help interpret the results and identify genes whose resistance is beginning to decline. This will also help to track changes in the pathogen's virulence levels.

### 3.3. Pathotypes Structure

Among the 160 isolates tested, 46 pathotypes were identified. The most numerous was the TBBB pathotype, represented by 30% of the tested isolates. It was present in all analyzed populations. 8.1% and 6.3% of the isolates represented the TBCB and RBBB pathotypes, respectively. The remaining pathotypes were represented by less than 5% of the isolates. The number of pathotypes and thus the diversity of the pathogen population increased in the following years. In the population collected in 2016, 12 pathotypes were identified, as well as 17 in the population collected in 2017, and 16 in the population from 2018. The population collected in 2019 was the most diverse and 21 different pathotypes were identified (Table 4).

**Table 4.** Virulence spectra of 46 pathotypes of *Blumeria graminis* f.sp. *avenae*.

	Pm1	Pm3	Pm6	Pm3 + 8	Pm2	Pm4	Pm5	Pm7	Pm7	Pm9	Pm10	Pm11	U A. Sterilis	U A. Strigosa	Number of Isolates				
															2016	2017	2018	2019	2016–2019
TBBB	+	+	+	+											18	14	13	3	48
TBCB	+	+	+	+								+			5	5	3		13
RBBB	+	+		+													2	8	10
TBBL	+	+	+	+									+			2	4	1	7
TBGB	+	+	+	+						+						1	4	1	6
NBCB	+		+															5	5
TBDB	+	+	+	+							+					4	1		5
TBJB	+	+	+	+						+	+					1	3	1	5
KBBB		+	+	+												4			4
TBCL	+	+	+	+								+	+		3		1		4
TBLB	+	+	+	+					+						4				4
KBCB		+	+	+								+				3			3
TBHB	+	+	+	+						+		+				2	1		3
LBDB	+										+							2	2
NBBB	+		+															2	2
NBBL	+		+										+				2		2
NBCG	+		+									+		+				2	2
NBDB	+		+								+				2				2
QBGG	+	+												+				2	2
TBDL	+	+	+	+							+		+		1	1			2
TBKB	+	+	+	+						+	+	+			2				2
TLBB	+	+	+	+	+													2	2
TLBL	+	+	+	+	+								+					2	2
DBBL			+										+					1	1
FBBB			+	+												1			1
FLBB			+	+	+													1	1
HBBB		+		+													1		1
KBBL		+	+	+									+		1				1
LBCL	+											+	+					1	1

Table 4. Cont.

	Pm1	Pm3	Pm6	Pm3 + 8	Pm2	Pm4	Pm5	Pm7	Pm7	Pm9	Pm10	Pm11	U <sub>A. Sterilis</sub>	U <sub>A. Strigosa</sub>	Number of Isolates						
															2016	2017	2018	2019	2016–2019		
NBCL	+		+									+	+					1		1	
NBDL	+		+								+		+					1		1	
NBJL	+		+							+	+		+						1		1
NBNL	+		+						+		+		+					1		1	
RBBG	+	+		+											+					1	1
RBCB	+	+		+								+								1	1
RBGB	+	+		+						+										1	1
RLBG	+	+		+	+										+					1	1
TBBG	+	+	+	+											+					1	1
TBCG	+	+	+	+								+			+					1	1
TBDQ	+	+	+	+							+		+	+						1	1
TBFB	+	+	+	+							+	+								1	1
TBHL	+	+	+	+						+		+	+							1	1
TBJG	+	+	+	+						+	+				+					1	1
TBJL	+	+	+	+						+	+		+							1	1
TLDL	+	+	+	+	+						+		+							1	1
TLJB	+	+	+	+	+					+	+									1	1

### 3.4. Diversity within and Distance between Populations

Different types of diversity parameters were calculated for individual populations. All the obtained results are presented in Table 5. These results clearly indicate that the differentiation of the *B.graminis* f.sp. *avenae* population in Poland increases year by year. The highest rates were observed for the population collected in 2019, which confirms its highest level of diversity.

Table 5. Diversity analysis of all powdery mildew isolates.

Parameter	2016	2017	2018	2019
No. of isolates	40	40	40	40
No. of different pathotypes	12	13	16	21
Gene diversity (Nei index $H_s$ ) equivalent to $ADW_m$ diversity	0.134	0.103	0.136	0.216
Simpson index $S_i$	0.758	0.828	0.853	0.916
Shannon normalized index $Sh$	0.514	0.579	0.635	0.754
Kosman index $KW_m$	0.164	0.129	0.168	0.300

The genetic distance calculated between all analyzed populations showed that the populations collected in 2016, 2017, and 2018 were the most similar to each other. The population collected in 2019 was the most different from all other populations.

The increase in the diversity of the *B.graminis* f.sp. *avenae* populations observed in 2016–2019 may be related to the weather changes taking place in these years (Table 6). The increase in the average temperature and high humidity had a significant impact on better wintering of spores and the passage of the full cycle of reproduction through the pathogen, which resulted in the emergence of new pathotypes.

**Table 6.** Meteorological data from the years and places of collection of the pathogen population [35].

Year	Location																			
	Choryń				Strzelce				Czesławice				Polanowice				Poland (Total)			
	2016	2017	2018	2019	2016	2017	2018	2019	2016	2017	2018	2019	2016	2017	2018	2019	2016	2017	2018	2019
Rainfall [mm]	608	668	373	393	751	832	520	388	698	612	479	531	745	702	569	639	666	733	489	556
Temperature [°C]	9.8	9.7	10.7	11.1	9.3	8.8	9.8	10.3	8.7	8.4	9.3	9.8	9.4	9.1	10	10.4	9.2	9	9.8	10.2
Wind velocity [m/s]	3.6	4	3.9	3.9	3.3	3.4	3.1	3.3	2.9	3	2.7	3	3.1	2.6	3.1	3.1	3.23	3.25	3.2	3.33
Insolation [h]	1823	1739	2225	2040	1840	1684	2170	2065	1872	1783	2143	2210	1750	1710	1949	1977	1821	1729	2122	2073

#### 4. Discussion

Assessing the level of virulence of pathogens is and will continue to be an integral part of breeding programs aimed at increasing the resistance of crops, as well as research focusing on the analysis of pathogenicity and virulence dynamics in pathogen populations [12,36]. Changes in virulence in the population and the speed of the appearance of new races of pathogens determine the possibility of using resistance genes in plant breeding. Therefore, it is important to observe the effectiveness of the resistance genes present in the cultivars [12,31,37].

The presented results on the frequency of virulence and comprehensiveness are a continuation of research on the dynamics of changes in the *B. graminis* f.sp. *avenae* populations in Poland since 2010 [33,38]. They showed that the diversity in the pathogen populations in Poland only slightly increases from year to year. This is confirmed by all the calculated differentiation parameters as well as the number of pathotypes identified in individual years. This number is growing successively from year to year. The number of pathotypes was two in 2010 and increased to eight in 2015 [33,38]. In recent years, this has ranged from 12 in 2016 to 21 in 2019. The increase in population diversity may be associated with better wintering of pathogen spores. The mild winters observed in recent years, as well as favorable weather conditions, favored the pathogen's survival, which allowed it to undergo a full cycle of sexual reproduction, and thus for the emergence of more diverse forms. Such a trend was noted by Tang et al. [5], who analyzed the impact of climate change on the pathogenicity of wheat powdery mildew. On the basis of long-term observations, they have shown that climate change contributes to the increase in powdery mildew epidemics, which may lead to an increase in the importance of powdery mildew as the main factor of the quality and quantity of wheat yield reduction.

Climate change also affects the spread of diseases to new geographic regions [1,4,39]. In recent years, powdery mildew symptoms were observed in China and in the Himalayan region [10,11], which allows the conclusion that climate change also affects *B. graminis* f.sp. *avenae*. These reports increase the need for continuous work on this pathogen, which will allow for the planning of effective oat crop protection strategies. Therefore, monitoring virulence in different regions of the world is very important and requires the unification of the method of conducting works so that it is possible to compare the obtained results.

The use of standardized nomenclature of pathogen isolates by various scientists allows for the comparison of works from different regions of the world and for drawing global conclusions regarding, for example, the pathogen's migration directions, which allows wise planning of plant protection strategies. Unified systems of nomenclature and pathogenicity description are currently carried out for many plant pathogens, for example: *Puccinia graminis* f. sp. *tritici* [40–43], *Puccinia triticina* [44–46], *Puccinia coronata* f. sp. *avenae* [47–50].

Until now, the evaluation of the *B. graminis* f.sp. *avenae* races was based on determining the isolate as virulent or avirulent in relation to the described resistance genes. Herrmann and Mohler [20] used the spores of the pathogen taken from a susceptible cultivar Pergamon. Hsam and Zeller [51] assessed the segregation of resistance used by the

isolate which was described as avirulent for the cultivar Mostyn. Herrmann and Roderick [52] described the isolate as infecting all cultivars from the UK. Mohler et al. [53] and Sánchez-Martín et al. [54] described the isolates only with symbols. Such descriptions can be misleading, especially if the isolate's response is not compared to the infection pattern of the control line. This kind of description provides only cursory information on the pathogen isolates used. Moreover, these results cannot be compared with each other due to the use of different control forms. In many studies on powdery mildew in oats, the characterization of the pathogen isolates is based on the presentation of the reactions of control genotypes to the isolates used in the experiment in a separate table. Hsam et al. [16,19] postulated the presence of resistance genes in oat cultivars based on the comparison of the reactions of the tested cultivars with the response of control forms. The characteristics of the isolates were presented as a table with a description of the resistant, sensitive or moderate reaction of the line to a given isolate. A similar way of presenting the level of virulence in isolates was used by Hsam et al. [32], when testing the segregation of resistance to *B. graminis* f.sp. *avenae* in oat populations. The use of letter code proposed in the present study will allow for a very simple presentation of the virulence of the used isolate without the need to present extensive tables. The control set proposed for the description of the *B. graminis* f.sp. *avenae* pathotypes contains all the oat powdery mildew resistance genes described so far. Additionally, it was supplemented with two genotypes identified by us as effective against powdery mildew. Moreover, in the available scientific literature there are many reports on the identification of other new, effective sources of resistance to powdery mildew [52,54,55]. These genotypes also could be included in the control set. The expansion of the control set with new genotypes will not disturb the developed system of nomenclature of *B. graminis* f.sp. *avenae* isolates.

To summarize, climate change in recent years has contributed to the spread of powdery mildew and an increase in the diversity of races of the pathogen. Our research has confirmed that the pathogen population changes from year to year and its monitoring allowed us to determine the effectiveness of the resistance genes used in breeding programs. In addition, monitoring changes in virulence and complexity can provide useful information for combining genes into pyramids to build long-term and comprehensive resistance. In our opinion, it is also necessary to standardize the nomenclature of *B. graminis* f.sp. *avenae* isolates. The code we propose will allow for the unification of the work carried out and for drawing global conclusions regarding the dynamics of changes in the pathogen's populations. It will also allow the monitoring of the emergence of pathotypes capable of breaking the most effective resistance genes.

**Author Contributions:** Conceptualization and methodology M.C., S.O., T.O. and A.K; validation, M.C. and T.O.; formal analysis, M.C. and S.O.; investigation, S.O. and T.O.; writing—original draft preparation, M.C. and S.O.; writing—review and editing, T.O. and A.K.; All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Data are available upon request from the corresponding authors.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Nazir, N.; Bilal, S.; Bhat, K.; Shah, T.; Badri, Z.; Bhat, F.; Wani, T.; Mugal, M.; Parveen, S.; Dorjry, S. Effect of Climate Change on Plant Diseases. *Artic. Int. J. Curr. Microbiol. Appl. Sci.* **2018**, *7*, 250–256. [[CrossRef](#)]
2. Grulke, N.E. The nexus of host and pathogen phenology: Understanding the disease triangle with climate change. *New Phytol.* **2011**, *189*, 8–11. [[CrossRef](#)] [[PubMed](#)]
3. Braun, U.; Cook, R.T.A.; Inman, A.J.; Shin, H.D. The taxonomy of the powdery mildew fungi. In *The Powdery Mildews: A Comprehensive Treatise*; APS Press: New York, NY, USA, 2002; pp. 13–55.

4. Baker, R.H.A.; Sansford, C.E.; Jarvis, C.H.; Cannon, R.J.C.; MacLeod, A.; Walters, K.F.A. The role of climatic mapping in predicting the potential geographical distribution of non-indigenous pests under current and future climates. *Agric. Ecosyst. Environ.* **2000**, *82*, 57–71. [[CrossRef](#)]
5. Tang, X.; Luo, Y.; Ma, Z.; Fan, J.; Zhou, Y.; Cao, X.; Xu, X.; Jiang, Y.; Luo, Y.; Ma, Z.; et al. Effects of Climate Change on Epidemics of Powdery Mildew in Winter Wheat in China. *Plant Dis.* **2017**, *101*, 1753–1760. [[CrossRef](#)]
6. Dean, R.; Van Kan, J.A.N.A.L.; Pretorius, Z.A.; Hammond-Kosack, K.E.I.M.E.; Di Pietro, A.; Spanu, P.D.; Rudd, J.J.; Dickman, M.; Kahmann, R.; Ellis, J.; et al. The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **2012**, *13*, 414–430. [[CrossRef](#)]
7. Schwarzbach, E.; Smith, I.M. *Erysiphe graminis* DC. In *European Handbook of Plant Diseases*; Smith, I.M., Dunez, J., Lelliot, R.A., Philips, D.H., Archer, S.A., Eds.; Blackwell: Kay County, OK, USA, 1988; pp. 259–261.
8. Roderick, H.W.; Jones, E.R.L.; Šebesta, J.; Sebesta, J. Resistance to oat powdery mildew in Britain and Europe: A review. *Ann. Appl. Biol.* **2000**, *136*, 85–91. [[CrossRef](#)]
9. Sebesta, J.; Kummer, M.; Roderick, H.W.W.; Hoppe, H.D.D.; Swierczewski, A.; Mueller, K.; Cervenka, J.; Swierczewski, A.; Muller, K. Breeding oats for resistance to rusts and powdery mildew in central Europe. *Ochr. Rostl.* **1991**, *27*, 229–238.
10. Xue, L.H.; Li, C.J.; Zhao, G.Q. First Report of Powdery Mildew Caused by *Blumeria graminis* on *Avena sativa* in China. *Plant Dis.* **2017**, *101*, 1954. [[CrossRef](#)]
11. Banyal, D.K.; Sood, V.K.; Singh, A.; Mawar, R. Integrated management of oat diseases in north-western Himalaya. *Range Manag. Agrofor.* **2016**, *37*, 84–87.
12. Traskovetskaya, V.; Gorash, A.; Liatukas, Ž.; Sauliak, N.; Ternovyi, K.; Babayants, O.; Ruzgas, V.; Leistrumaitė, A. Virulence and diversity of the blumeria graminis f. sp. tritici populations in lithuania and Southern Ukraine. *Zemdirbyste* **2019**, *106*, 107–116. [[CrossRef](#)]
13. Chong, J.; Leonard, K.J.; Salmeron, J.J. A North American System of Nomenclature for *Puccinia coronata* f. sp. *avenae*. *Plant Dis.* **2000**, *84*, 580–585. [[CrossRef](#)]
14. Roelfs, A.P.; Martens, J.W. An International System of Nomenclature for *Puccinia graminis* f. sp. *Tritici*. *Phytopathology* **1988**, *78*, 526–533. [[CrossRef](#)]
15. Long, D.L.; Kolmer, J.A. A north American system of nomenclature for *Puccinia recindita* f.sp. *tritici*. *Phytopathology* **1989**, *79*, 525–529. [[CrossRef](#)]
16. Hsam, S.L.K.; Pederina, E.; Gorde, S.; Zeller, F.J. Genetic studies of powdery mildew resistance in common oat (*Avena stativa* L.). II. Cultivars and breeding lines grown in Northern and Eastern Europe. *Hereditas* **1998**, *129*, 227–230. [[CrossRef](#)]
17. Okoń, S.; Paczos-Grzeda, E.; Ociepa, T.; Koroluk, A.; Sowa, S.; Kowalczyk, K.; Chrza, M. *Avena sterilis* L. Genotypes as a Potential Source of Resistance to Oat Powdery Mildew. *Plant Dis.* **2016**, *100*, 2145–2151. [[CrossRef](#)] [[PubMed](#)]
18. Okoń, S.; Kowalczyk, K. Screening oat landraces for resistance to *Blumeria graminis* f. sp. *avenae*. *J. Plant Pathol.* **2020**, *102*, 893–898. [[CrossRef](#)]
19. Hsam, S.L.K.; Peters, N.; Paderina, E.V.; Felsenstein, F.; Oppitz, K.; Zeller, F.J. Genetic studies of powdery mildew resistance in common oat (*Avena sativa* L.). I. Cultivars and breeding lines grown in Western Europe and North America. *Euphytica* **1997**, *96*, 421–427. [[CrossRef](#)]
20. Herrmann, M.H.; Mohler, V. Locating two novel genes for resistance to powdery mildew from *Avena byzantine* in the oat genome. *Plant Breed.* **2018**, *137*, 832–838. [[CrossRef](#)]
21. Okoń, S.M.; Ociepa, T. Effectiveness of new sources of resistance against oat powdery mildew identified in *A. sterilis*. *J. Plant Dis. Prot.* **2018**, *125*, 1–6. [[CrossRef](#)]
22. Mains, E.B. Inheritance of resistance to powdery mildew, *Erysiphe graminis tritici*, in wheat. *Phytopathology* **1934**, *24*, 1257–1261.
23. Dreiseitl, A.; Kosman, E. Virulence phenotypes of *Blumeria graminis* f. sp. *hordei* in South Africa. *Eur. J. Plant Pathol.* **2015**, *136*, 113–121. [[CrossRef](#)]
24. Kosman, E. Difference and diversity of plant pathogen populations: A new approach for measuring. *Phytopathology* **1996**, *86*, 1152–1155.
25. Kosman, E.; Leonard, K.J. Conceptual analysis of methods applied to assessment of diversity within and distance between populations with asexual or mixed mode of reproduction. *New Phytol.* **2007**, *174*, 683–696. [[CrossRef](#)]
26. Herrmann, A.; Löwer, C.; Schachtel, G. A new tool for entry and analysis of virulence data for plant pathogens. *Plant Pathol.* **1999**, *48*, 154–158. [[CrossRef](#)]
27. Schachtel, G.A.; Dinoor, A.; Herrmann, A.; Kosman, E. Comprehensive Evaluation of Virulence and Resistance Data: A New Analysis Tool. *Plant Dis.* **2012**, *96*, 1060–1063. [[CrossRef](#)] [[PubMed](#)]
28. Okoń, S.M. Identification of powdery mildew resistance genes in Polish common oat (*Avena sativa* L.) cultivars using host-pathogen tests. *Acta Agrobot.* **2012**, *65*, 63–68. [[CrossRef](#)]
29. Okoń, S.; Ociepa, T.; Paczos-Grzeda, E.; Kowalczyk, K. Analysis of the level of resistance of Polish oat cultivars (*Avena sativa* L.) to powdery mildew (*Blumeria graminis* DC. f. sp. *avenae* Em. Marchal.). *Agron. Sci.* **2016**, *61*, 51–60.
30. Kowalczyk, K.; Hsam, S.L.K.; Zeller, F.J. Identification of oat powdery mildew resistance 2 (OMR2) and Polish common oat (*Avena sativa* L.) cultivars. In *Workshop “Resistance of Cereals to Biotic Stresses”*; IHAR: Radzików, Poland, 2004; pp. 122–125.
31. Okoń, S.M. Effectiveness of resistance genes to powdery mildew in oat. *Crop Prot.* **2015**, *74*, 48–50. [[CrossRef](#)]

32. Hsam, S.L.K.; Mohler, V.; Zeller, F.J. The genetics of resistance to powdery mildew in cultivated oats (*Avena sativa* L.): Current status of major genes. *J. Appl. Genet.* **2014**, *55*, 155–162. [CrossRef]
33. Cieplak, M.; Terlecka, K.; Ociepa, T.; Zimowska, B.; Okoń, S. Virulence Structure of *Blumeria graminis* f. sp. *avenae* Populations in Poland across 2014–2015. *Plant Pathol. J.* **2021**, *37*, 115–123. [CrossRef]
34. Ociepa, T.; Okoń, S.; Nucia, A.; Leśniowska-Nowak, J.; Paczos-Grzęda, E.; Bisaga, M. Molecular identification and chromosomal localization of new powdery mildew resistance gene Pm11 in oat. *Theor. Appl. Genet.* **2020**, *133*, 179–185. [CrossRef]
35. Statistics Poland/Databases. Available online: <https://stat.gov.pl/en/databases/> (accessed on 10 September 2021).
36. Paczos-Grzęda, E.; Sowa, S. Virulence Structure and Diversity of *Puccinia coronata* f. sp. *avenae* P. Syd. & Syd. in Poland During 2013 to 2015. *Plant Dis.* **2019**, *103*, 1559–1564.
37. Babayants, O.V.; Babayants, L.T.; Traskovetskaya, V.A.; Gorash, A.F.; Saulyak, N.I.; Galaev, A.V. Race Composition of *Blumeria graminis* (DC) Speer f. sp. *tritici* in the South of Ukraine and Effectiveness of Pm-genes in 2004–2013. *Cereal Res. Commun.* **2015**, *43*, 449–458. [CrossRef]
38. Okoń, S.M.; Ociepa, T. Virulence structure of the *Blumeria graminis* DC. f. sp. *avenae* populations occurring in Poland across 2010–2013. *Eur. J. Plant Pathol.* **2017**, *149*, 711–718. [CrossRef]
39. Etterson, J.R.; Shaw, R.G. Constraint to adaptive evolution in response to global warming. *Science* **2001**, *294*, 151–154. [CrossRef]
40. Dumbadze, R.Z.; Sikharulidze, Z.V. Virulence Structure of the Wheat Stem Rust Population in Georgia. *Int. J. Agric. Innov. Res.* **2016**, *4*, 5.
41. Fetch, T.; Zegeye, T.; Park, R.F.; Hodson, D.; Wanyera, R. Detection of wheat stem rust races TTHSK and PTKTK in the Ug99 race group in Kenya in 2014. *Plant Dis.* **2016**, *100*, 1495. [CrossRef]
42. Skolotneva, E.S.; Kosman, E.; Patpour, M.; Kelbin, V.N.; Morgounov, A.I.; Shamanin, V.P.; Salina, E.A. Virulence Phenotypes of Siberian Wheat Stem Rust Population in 2017–2018. *Front. Agron.* **2020**, *2*, 6. [CrossRef]
43. Rsaliyev, A.; Yskakova, G.; Maulenbay, A.; Zakarya, K.; Rsaliyev, S. Virulence and race structure of *Puccinia graminis* f. sp. *tritici* in Kazakhstan. *Plant Prot. Sci.* **2020**, *56*, 275–284. [CrossRef]
44. Nemati, Z.; Mostowfzadeh-Ghalamfarsa, R.; Dadkhodaie, A.; Mehrabi, R.; Steffenson, B.J. Virulence of leaf rust physiological races in Iran from 2010 to 2017. *Plant Dis.* **2020**, *104*, 363–372. [CrossRef]
45. Boshoff, W.H.P.; Labuschagne, R.; Terefe, T.; Pretorius, Z.A.; Visser, B. New *Puccinia triticina* races on wheat in South Africa. *Australas. Plant Pathol.* **2018**, *47*, 325–334. [CrossRef]
46. Mantovani, P.; MacCafferri, M.; Tuberosa, R.; Kolmer, J. Virulence phenotypes and molecular genotypes in collections of *Puccinia triticina* from Italy. *Plant Dis.* **2010**, *94*, 420–424. [CrossRef] [PubMed]
47. Leonard, K.J.; Paul, S.; Martinelli, J.A. Virulence of Oat Crown Rust in Brazil and Uruguay. *Plant Dis.* **2005**, *89*, 802–808. [CrossRef]
48. Carson, M.L. Additional Sources of Broad-Spectrum Resistance to *Puccinia coronata* f. sp. *avenae* from Canadian Accessions of *Avena barbata*. *Plant Dis.* **2010**, *94*, 1405–1410. [CrossRef]
49. Paczos-Grzęda, E.; Sowa, S.; Boczkowska, M.; Langdon, T. Detached Leaf Assays for Resistance to Crown Rust Reveal Diversity Within Populations of *Avena sterilis*. *Plant Dis.* **2019**, *103*, 832–840. [CrossRef]
50. Paczos-Grzęda, E.; Sowa, S.; Koroluk, A.; Langdon, T. Characteristics of Resistance to *Puccinia coronata* f. sp. *avenae* in *Avena fatua*. *Plant Dis.* **2018**, *102*, 2616–2624. [CrossRef] [PubMed]
51. Hsam, S.L.K.; Zeller, F.J. Chromosomal location of genes for resistance to powdery mildew in cultivated oat (*Avena sativa* L.). 1. Gene Eg-3 in the cultivar ‘Mostyn’. *Plant Breed.* **1998**, *178*, 177–178. [CrossRef]
52. Herrmann, M.; Roderick, H.W. Characterisation of new oat germplasm for resistance to powdery mildew. *Euphytica* **1996**, *89*, 405–410. [CrossRef]
53. Mohler, V.; Zeller, F.J.; Hsam, S.L.K. Molecular mapping of powdery mildew resistance gene Eg-3 in cultivated oat (*Avena sativa* L. cv. Rollo). *J. Appl. Genet.* **2012**, *53*, 145–148. [CrossRef]
54. Sánchez-Martín, J.; Rubiales, D.; Prats, E. Resistance to powdery mildew (*Blumeria graminis* f. sp. *avenae*) in oat seedlings and adult plants. *Plant Pathol.* **2011**, *60*, 846–856. [CrossRef]
55. Okoń, S.; Ociepa, T.; Paczos-Grzęda, E.; Ladizinsky, G. Evaluation of resistance to *Blumeria graminis* DC f. sp. *avenae*, in *Avena murphyi* and *A. magna* genotypes. *Crop Prot.* **2018**, *106*, 177–181. [CrossRef]