Seasonal Patterns of Spore Dispersal of *Phaeosphaeria* spp. and *Stagonospora* spp.

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ABSTRACT

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The spatial and temporal patterns of discharge and dissemination of airborne spores of *Phaeosphaeria* spp. and *Stagonospora* spp. were studied. Both ascospores and pycnidiospores of the pathogens were deposited at various densities on microscope slides used as spore samplers. The maximum deposition of the spores was observed during the period of August to October. A multiple regression analysis was used to determine which weather factors significantly explained the variation measured in the numbers of ascospores that settled on microscope slides. Rainfall, air temperature, and relative air humidity were influential in the release of *Phaeosphaeria* spp. ascospores into the air. The amount of airborne ascospores was a function of the variables and remained largely under their control. The liberation of ascospores was favored by air temperature above 0°C, rainfall greater than 1 mm, and high relative humidity. The range of atmospheric conditions stimulating air dispersal of ascospores was wider than that for pycnidiospores. Pycnidiospores were sampled only during rainy days. Their release was affected adversely by air temperature below 5°C. Multiple regression models based on weather data were developed and verified for their predictive ability and accuracy by jackknife and cross-validation procedures, as well as by comparisons of observed and predicted mean numbers of deposited ascospores per microscope slide after a substitution of each period data set with a set of data of the other respective time interval. The numbers of airborne ascospores predicted by the regression models were in a good agreement with the observed values. The jackknife and cross-validation techniques allowed use of the limited data sets for both the parameter estimation and validation processes in a development of simulation models. The airborne inoculum appeared to be omnipresent over cereal areas year round, except during periods with freezing temperatures and a snow cover. Such an omnipresence of inoculum of the pathogens poses a danger to crops and could be of importance in the epidemiology of *Stagonospora* (= *Septoria*) blotches under the climatic conditions of central Poland.

Additional keywords: air dispersal, *Phaeosphaeria* (= *Leptosphaeria*) *nodorum*, *P. avenaria* f. sp. *triticea*, *Stagonospora* (= *Septoria*) *nodorum*, *S. avenae* f. sp. *triticea*

Phaeosphaeria nodorum (E. Müller) Hedjaroude, syn. *Leptosphaeria nodorum* E. Müller (anamorph: *Stagonospora nodorum* (Berk.) Castellani & Germano, syn. *Septoria nodorum* Berk.) and *P. avenaria* (G.F. Weber) O. Eriksson f. sp. *triticea*, syn. *L. avenaria* (G.F. Weber) f. sp. *triticea* T. Johnson (anamorph: *Septoria avenae* A.B. Frank f. sp. *triticea* T. Johnson) incite *Stagonospora* (= *Septoria*) blotches of wheat (*Triticum aestivum* L.) and triticale ($\times$ *Triticosecale* Wittmack) in Poland (1,2). Grain yield losses in the two cereal small grains afflicted by *Stagonospora nodorum* blotch may reach 53% (11). A mild, natural infection of triticale leaves and heads by *S.*

nodorum resulted in 10% grain yield loss (3). The diseases caused by phytopathogenic *Phaeosphaeria* spp. in Poland are important on triticale due to an expansion in planting to about 700,000 ha, at present, and a large area of spring and winter wheats (about 2.5 million ha). The fungi overwinter in seed, plant residues, graminaceous weeds, cereal seedlings, and volunteer plants. *P. nodorum* in wheat and in triticale is disseminated via infected seed, airborne plant debris, ascospores, and pycnidiospores (4,11,23–25,33).

The sexual and asexual reproductive organs of these fungi require different weather conditions for the release and dissemination of spores. The release of spores into the atmosphere also involves biological factors. Concentrations of fungal spores in the air are influenced by their formation rate, release, and rate of deposition. Weather factors, especially precipitation, affect the growth of fungi and the amount and timing of production of spores, their release from the fruiting bodies, dispersal, and deposition. According to Ingold (19), in the majority of *Ascomycetes* the ascus is a turgid cell that bursts in a regular man-

ner, violently liberating its ascospores. These spores are usually actively discharged to a distance of 0.5 to 2.0 cm, then dispersed by air currents (11,15,30).

Spores of *Stagonospora* spp. are formed in pycnidia (17,26,28). A pycnidial mucilage absorbs water (5,31), resulting in the extrusion of a cirrus of pycnidiospores through the ostiole. On wetting, the mucilage dissolves to leave a pycnidiospore suspension in a thin film of water on the host surface. Pycnidiospores are dispersed by rainsplash about 1 m horizontally and to a height of up to 2 m, or they become airborne in splash microdroplets (6,7,36).

This paper describes the dissemination patterns of spores of *Phaeosphaeria* spp. and *Stagonospora* spp. in air studied over a 3-year period. The objective of the study was to determine under which weather conditions and times of year spores are released and dispersed into the air. A secondary objective was to arrive at a mathematical description of the relationships between rainfall, air temperature, and relative air humidity and the number of ascospores of *Phaeosphaeria* spp. dispersed in the air. Preliminary data of the study have been published (13).

MATERIALS AND METHODS

Spores were sampled by passive deposition onto microscope slides covered with petroleum jelly and attached to wooden sticks at a height of 0.7 m above the soil surface. Two slides were mounted on each stick (herein called a spore sampler), one vertically and the other horizontally. Such mounting of slides has been considered the most effective way to sample spores under changing weather conditions (10).

In 1991 and 1992, 4 spore samplers were located 2, 25, and 50 m from a triticale plot (Plot 1). At the same time, spores were sampled from a second triticale plot (Plot 2), but only at a distance of 2 m. The sampling area of each microscope slide was 19 cm²; therefore, at each distance the combined spore sampling area was 152 cm². The spore samplers were arranged in a line extending from the wide axis of the experimental plots. Plots were separated by a 100-m area planted to grasses and faba beans. Plots were surrounded on the west side by rye and grass fields, on the north and south sides by faba bean and grass fields, and on the east side by bushes and grasses. Each plot was 20 m long and 1 m wide and arranged longitudinally in a west-east direction. Wind direction in the area is usually from the west to east. Both plots

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Slides were examined under a light microscope at 100× magnification. The morphology of ascospores of *Phaeosphaeria* spp. and pycnidiospores of *Stagonospora*

Figure 1 consists of three vertically stacked panels (A, B, and C) sharing a common x-axis representing sampling dates from 1/2 to 12/31. Panel A shows the number of spores per slide per day for three locations: Fence (black bars), Plot 1 (dark gray bars), and Plot 2 (light gray bars). The y-axis ranges from 0 to 6. A legend at the top left identifies the locations. Panel B shows relative humidity (%) as a line graph, with the y-axis ranging from 70 to 100. A legend at the top left identifies the line as 'Relative humidity'. Panel C shows rainfall (mm) as a bar graph and temperature (°C) as a line graph. The y-axis for rainfall ranges from 0 to 9, and for temperature from -5 to 25. A legend at the top left identifies the bars as 'Rainfall' and the line as 'Temperature'. All three panels have a top x-axis with sampling dates (month/day) and a bottom x-axis with day numbers (1 to 31). Arrows at the top of each panel indicate specific sampling events.

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The viability of the airborne spores was determined by recording their germination directly on petroleum jelly on the slides, on oxgall (4) and triticale meal agars, respectively, in petri plates exposed horizontally to the air currents for several hours. Four plates per plot were exposed.

Weather variables such as rainfall, air temperature, and relative air humidity (Fig. 1B,C) were recorded at the meteorological station in Radzików. The station was located approximately 250 m from the experimental field and spore samplers.

Relationships between the deposition of airborne ascospores and weather variables. Regression analyses were used to explore, measure, and model the relationships between the numbers of *Phaeosphaeria* spp. ascospores sampled each day and three weather variables. Data collected during the periods of 16 July to 6 December 1991 and 9 June to 30 November 1992 (i.e., the periods with the maximum deposition of ascospores) were used for the regression analyses (Tables 1 and 2).

Because the ascospore counts and rainfall heights often had zero values and also high values, they showed a skewed distribution. Both of the variables were thus transformed with the square root (sqrt) transformation ($y = \text{sqrt}[\text{number of ascospores}]$, $x_2 = \text{sqrt}[\text{rainfall in mm}]$).

The number of ascospores sampled per slide was regressed on weather variables: mean daily air temperature, daily rainfall, and mean daily relative humidity. After fitting the initial exploratory model (Table 1), a more complex multiple regression model, which combined effects of weather variables and their multiplicative interaction terms, was developed: $E(y) = a + b_1x_1 + b_2x_1^2 + b_3x_2 + b_4x_2^2 + b_5x_3 + b_6x_3^2 + b_7x_1x_2 + b_8x_1x_3 + b_9x_2x_3 + b_{10}x_1x_2x_3$, where $E(y)$ was the expected number of ascospores, x and x^2 were linear and non-linear components in the response of the number of ascospores to the specific weather variable, and a , b_1 , and b_2 were the intercept and regression coefficients, respectively. In the model, x_1 (air temperature), x_2 (sqrt [rainfall]), and x_3 (relative air humidity) were the weather variables studied. The model was checked for meeting the residual and normality requirements. Then individual terms were tested for significance using the Linear Regression module of STATISTICA for Windows 5.0 (StatSoft, Inc., Tulsa, OK) according to the following rules: (i) interaction terms were tested first, (ii) lower order terms were not dropped if the higher order ones in that variable were significant, and (iii) if an interaction term was significant, its individual variables of the same order were included in the model.

Model validation. The predictive ability and accuracy of the regression models were tested according to the techniques proposed by Jones and Carberry (20) for

the development and validation of simulation models. The 1991 and 1992 databases, composed of the dependent variable values and the weather parameters which entered the regression model, were divided into 13 and 16 data sets, respectively, of size 11 days. The first-order jackknife estimation of 5 parameters, which entered the models, was done for each year on all the data, and on data of $n - 1$ data sets (i.e., when 1 out of 13 or 16 data sets, respectively, was omitted at a time). The partial values of the estimated parameters of the regression models for the 13 and 16 sets of data are given in Tables 3 and 4. Using formulas given in Jones and Carberry (20), overall estimates (P), the mean partial values (P[.]), the first-order standard estimates (P*), and the jackknife standard errors (SE*), as well as the cross-validation estimates, were calculated and tabulated (Tables 3 and 4).

RESULTS

Ascospores. Single ascospores appeared to be the unit of airborne dissemination. The spores were usually uniformly spread over the microscope slides. In 1991, the first ascospores on spore samplers were detected by 21 March (Fig. 1A). The number of ascospores gradually increased to a maximum in mid-September (Fig. 1A). However, dry periods in the last week of August and the beginning of September of 1990 and 1991 reduced the numbers of ascospores sampled on the slides (Fig. 1A). A dramatic reduction in the air density of ascospores in July and August of 1992 (Fig. 2A) was correlated with a prolonged drought and high temperatures. Therefore, in comparison to 1990 and 1991, the deposition pattern in 1992 was noticeably distorted. (Fig. 2A; 13).

Ascospores were liberated from pseudothecia and dispersed mainly during rainy

Table 1. Correlations and partial regression coefficients between various weather variables and the number of *Phaeosphaeria* spp. ascospores sampled on microscope slides in 1991 and 1992

	1991 ^x	1992
Mean air temperatures (°C)	11.9	13.5
Sqrt ^y ascospores—temperature correlation	0.257*** ^z	0.309**
Percentage variance explained (R^2)	6.6	9.6
Partial correlation coefficient		
Temperature	0.241**	0.287**
(temperature) ²	-0.203***	-0.306**
Sqrt ascospores—sqrt rainfall correlation	0.567***	0.445***
Percentage variance explained (R^2)	32.2	19.8
Partial correlation coefficient		
Sqrt rainfall	0.476***	ns
Rainfall	-0.336***	ns
Sqrt ascospores—relative humidity correlation	0.326***	0.311**
Percentage variance explained (R^2)	10.6	9.7
Partial correlation coefficient		
relative humidity	0.275***	ns
(relative humidity) ²	-0.265***	ns
Number of samples	143	175

^x Sampling dates (month/day) for 1991 and 1992 were 16 July and 6 December, and 9 June and 30 November, respectively.

^y Sqrt = square root transformation.

^z ***, ** = coefficients significant at $P < 0.01$ and $P < 0.05$, respectively; ns = not significant.

Table 2. Multiple correlation coefficients (R) and partial regression (b) between numbers of *Phaeosphaeria* spp. ascospores sampled on microscope slides and weather variables in 1991 and 1992

	1991 ^w		1992	
	R^x	b	R	b
Temperature ^y	0.399*** ^z	0.1139	0.294***	0.0645
Temperature ²	-0.287***	-0.0033	-0.222***	-0.0018
Square root rainfall	0.431***	0.8621	0.291***	2.0106
Rainfall, mm	-0.321***	-0.2201	ns	ns
Relative humidity, %	0.339***	0.0457	0.244***	0.0212
Relative humidity ²	ns	...	ns	...
Square root rainfall × temperature	ns	...	ns	...
Square root rainfall × relative humidity	ns	...	-0.273***	-0.0201
Temperature × relative humidity	ns	...	ns	...
Number of samples	143		175	

^w Sampling dates (month/day) in 1991 and 1992 were 16 July and 6 December, and 9 June and 30 November, respectively.

^x Overall R values for square root ascospores – weather variables were 0.675*** and 0.585*** in 1991 and 1992, respectively. Percentage variance explained (R^2) was 45.5 and 34.2 for 1991 and 1992, respectively. Intercept-values were -3.1328 and -1.8798 for 1991 and 1992, respectively.

^y Mean air temperature (°C) was 11.9 and 13.5 in 1991 and 1992, respectively.

^z ***, ** = coefficients significant at $P < 0.01$ and $P < 0.05$, respectively; ns = not significant.

days. A rainfall of only about 1 mm or a prolonged dew period were sufficient to moisten pseudothecia to a level that stimulated a liberation and discharge of ascospores.

Ascospores were liberated and disseminated whenever the air temperature was above 0°C (Fig. 1A,C). A maximum daily air temperature above 25°C usually reduced ascospore dispersal. Temperatures as high as 37°C occurred in the summer of 1992. These air temperatures were associated with reduction in numbers of spores by about 30% in July, 80% in August, 50% in September, and distorted the seasonal pattern of spore dispersal in comparison to 1990 and 1991 (Fig. 2A). Infrequent but heavy rainfalls during that period of high temperatures did not increase the number of ascospores in the air. This finding indicates that high temperature hampered the production of ascospores in pseudothecia. In late August and early September 1992, a sudden drop in air temperature combined with more frequent rains increased the ascospore density in the air. However, this increase again was terminated by dry conditions during the second and third weeks of September and the beginning of October

(Fig. 2A). Lower air temperature also adversely influenced the number of ascospores in the air during the first and fourth weeks of April, the second week of September, the third week of October, and the second week of November 1991 (Figs. 1A,C, 2A). In the other weeks of October and November 1991, the numbers of ascospores on the slides were fairly constant, as well as in the other years of the study (Fig. 2A). A clear-cut drop in the ascospore deposition was noticed with an onset of freezing temperatures (i.e., mid-December 1990, the end of November 1991, and mid-November in 1992; Fig. 2A). Freezing temperatures and a snow cover completely stopped the dispersal of ascospores from the end of January until about 21 March 1991 (Fig. 1A,C).

The average numbers of ascospores/slide/day for 1990, 1991, and 1992

were 0.46, 0.39, and 0.16, respectively. The average number for 3 years was 0.32 ascospores sampled per slide per day. Mean numbers of ascospores/slide/day sampled at 3 distances from the line source of inoculum were not significantly different within a year. These numbers were 0.68 and 0.21 at 2 m, 0.65 and 0.15 at 25 m, and 0.56 and 0.17 at 50 m in 1991 and 1992, respectively. The striking finding was that a rather small number of ascospores were sampled per slide, even at peak times.

The germinability of the airborne ascospores and pycnidiospores was recorded (Fig. 1A,B). Spores germinated directly on petroleum jelly during the frost-free months from the beginning of June until the occurrence of freezing temperatures. Spore germination was not usually observed during periods with low or no rainfall (Fig. 1A). In comparison to asco-

Table 4. Cross-validation estimates on all data analyzed in 1991 and 1992

	1991	1992
S^2_0 (overall prediction variance)	0.258	0.161
S^2_{CV} (cross-validation prediction variance)	0.286	0.186
R^2_0 (overall determination coefficient)	0.486	0.347
R^2_{CV} (cross-validation determination coefficient)	0.399	0.246

Table 3. Jackknife estimates on all data analyzed in 1991 and 1992, respectively, and on resampled data sets

No.	Partial values for sets of data	Intercept	Temperature	(Temperature) ²	Sqrt ^z (rainfall)	Rainfall	Relative humidity
1991							
1.	P ₍₁₎	-3.3693	0.1227	-0.0035	0.8422	-0.2072	0.0370
2.	P ₍₂₎	-2.8838	0.1076	-0.0031	0.7871	-0.1903	0.0322
3.	P ₍₃₎	-3.2503	0.1148	-0.0033	0.8837	-0.2392	0.0360
4.	P ₍₄₎	-3.0453	0.1080	-0.0032	0.7260	-0.1760	0.0341
5.	P ₍₅₎	-2.9584	0.1157	-0.0034	0.8506	-0.2176	0.0329
6.	P ₍₆₎	-2.6554	0.1067	-0.0031	0.9660	-0.2520	0.0295
7.	P ₍₇₎	-3.0343	0.1017	-0.0028	0.8213	-0.2132	0.0339
8.	P ₍₈₎	-3.1875	0.1157	-0.0034	0.9116	-0.2358	0.0352
9.	P ₍₉₎	-3.4676	0.1236	-0.0036	0.8277	-0.2121	0.0381
10.	P ₍₁₀₎	-3.1863	0.1167	-0.0034	0.9584	-0.2514	0.0351
11.	P ₍₁₁₎	-3.0178	0.1115	-0.0032	0.9530	-0.2320	0.0331
12.	P ₍₁₂₎	-3.2664	0.1125	-0.0033	0.8460	-0.2153	0.0366
13.	P ₍₁₃₎	-3.3767	0.1235	-0.0036	0.8513	-0.2182	0.0370
P (overall estimates)		-3.1328	0.1139	-0.0033	0.8621	-0.2201	0.0347
P(.) (mean partial values)		-3.1307	0.1139	-0.0033	0.8635	-0.2206	0.0347
P* (first order estimates)		-3.1577	0.1135	-0.0033	0.8443	-0.2143	0.0350
SE* (jackknife standard errors)		0.7540	0.0228	0.0008	0.2331	0.0750	0.0080
1992							
1.	P ₍₁₎	-1.6920	0.0592	-0.0017	1.9815	0.0193	-0.0197
2.	P ₍₂₎	-1.5914	0.0620	-0.0019	1.0183	0.0183	-0.0096
3.	P ₍₃₎	-1.8329	0.0656	-0.0019	1.9672	0.0207	-0.0197
4.	P ₍₄₎	-2.0447	0.0684	-0.0020	2.3289	0.0229	-0.0236
5.	P ₍₅₎	-2.0150	0.0669	-0.0019	2.0123	0.0225	-0.0201
6.	P ₍₆₎	-1.8072	0.0632	-0.0018	1.9317	0.0205	-0.0193
7.	P ₍₇₎	-1.8313	0.0712	-0.0021	2.0575	0.0205	-0.0207
8.	P ₍₈₎	-2.2917	0.0650	-0.0017	2.4181	0.0255	-0.0244
9.	P ₍₉₎	-1.8816	0.0607	-0.0017	2.3598	0.0215	-0.0242
10.	P ₍₁₀₎	-1.9007	0.0641	-0.0019	1.9782	0.0215	-0.0198
11.	P ₍₁₁₎	-2.0937	0.0652	-0.0018	2.0058	0.0234	-0.0201
12.	P ₍₁₂₎	-1.7935	0.0644	-0.0019	1.9169	0.0203	-0.0191
13.	P ₍₁₃₎	-1.9012	0.0687	-0.0020	2.0543	0.0211	-0.0207
14.	P ₍₁₄₎	-1.9075	0.0715	-0.0020	2.0290	0.0209	-0.0203
15.	P ₍₁₅₎	-1.5438	0.0506	-0.0015	2.3594	0.0186	-0.0236
16.	P ₍₁₆₎	-1.9349	0.0608	-0.0017	1.9522	0.0222	-0.0195
P (overall estimates)		-1.8798	0.0645	-0.0018	2.0106	0.0212	-0.0201
P(.) (mean partial values)		-1.8789	0.0642	-0.0018	2.0232	0.0212	-0.0203
P* (first order estimates)		-1.8924	0.0691	-0.0020	1.8210	0.0211	-0.0180
SE* (jackknife standard errors)		0.6942	0.0191	0.0005	1.1936	0.0069	0.0127

^z Sqrt = square root transformation.

spores, the germinability of pycnidiospores was more adversely affected by the rainless weather (Fig. 1A,B). Triticale straw bundles were a long-lasting and effective source of ascospores of *P. nodorum* and *P. avenaria* f. sp. *triticea* throughout the study (Fig. 1A; 13). Very few pycnidiospores were sampled at this site until mid-October 1991 (Fig. 1B).

Pycnidiospores. Pycnidiospores and pieces of pycnidia, empty or with pycnidiospores, and clumps of pycnidiospores were observed on the slides. In order to facilitate the counting of spores, a slight amount of water with a detergent was used to resuspend the clumps. In 1990, only a preliminary record was prepared, without a regular counting of pycnidiospores on slides. The data from that year are included solely to demonstrate that the spores were present in the air (Fig. 2B). In 1991 and 1992, airborne pycnidiospores of *S. nodorum* were first observed on slides by the end of April (Fig. 2B). Their numbers increased considerably by mid-August 1991 and by the end of August 1992. After that, the numbers of pycnidiospores in both years remained quite high until the air temperature was close to or below 0°C; i.e., the end of November 1991 and mid-December 1992 (Figs. 1B,C, 2B). Few spores were sampled during rainless weeks, e.g., the second weeks of July and September 1991 (Fig. 1B,C). Since the spores were present on the crop during these weeks, the lack of rain is the only explanation for their absence in the air.

Pycnidiospore release was also adversely influenced by low air temperature. At the end of October and early November 1991, when the air temperature was low, spores were not found on slides in spite of rains and high relative humidity (Fig. 1B,C). Air temperature below 5°C stopped the liberation of pycnidiospores completely. Without a rain event, the air humidity did not by itself exert a noticeable effect on the presence of pycnidiospores in the air. In general, the patterns of pycnidiospore dispersal in 1991 and 1992 were similar. No attempt was made to regress the pycnidiospore number on weather variables because of their infrequent and rather erratic settling on the microscope slides during the sampling years.

The average numbers of pycnidiospores sampled daily on a microscope slide were 6.32 and 2.11 for 1991 and 1992, respectively. The lower number of pycnidiospores sampled on slides in 1992 was a consequence of a prolonged summer drought in that year. The average number for 2 years was 3.79 pycnidiospores/slide/day. The average number of pycnidiospores sampled daily per slide was about 12-fold larger (3.79 versus 0.32) than the average number of ascospores sampled per day (Fig. 2A).

Relationships between the deposition of airborne ascospores and weather

variables. Correlation coefficients and the percent variance of the ascospore data, explained for each period by the multiple regression equations assembled for individual weather variables, are presented in Table 1. Air temperature and the numbers of deposited ascospores showed rather weak associations, which accounted solely for 6.6 and 9.6% of explained variance in 1991 and 1992, respectively (Table 1).

Among the weather variables analyzed, rainfall showed the largest effect on the air density of ascospores. The regression model established for the latter relationship explained the largest percentages of variance, which were 32.8 and 19.8% for 1991 and 1992, respectively (Table 1).

Significant associations between air relative humidity and numbers of deposited ascospores were established for the sampling periods. The relationships explained 10.6 and 9.7% of variance for the first and second year of the study, respectively, and were the second in ranking to individually influence spore deposition on microscope slides (Table 1).

The multiple regression models established for 1991 and 1992 combined effects of all weather variables and their interactions. They corroborated and provided a better fit for the relationships established by the models designed for individual variables. The multiple determination (R^2)

coefficients calculated by the latter model were considerably larger (Table 2). They showed that the 34.2 to 45.5% variation in the number of sampled ascospores could be explained by the amount of rainfall, the air temperature, the relative air humidity, and the second degree multiplicative interaction of square root of rainfall by relative air humidity (Table 2).

The influence of air temperature on numbers of ascospores settled on microscope slides was significant in both 1991 and 1992, but more pronounced in 1991 (Table 2). This variable in its squared form was inversely associated with numbers of ascospores (Table 2). The effect of rainfall on the ascospore dispersal was the largest among the weather variables studied. A direct, positive association between the square root of rainfall and numbers of airborne ascospores was observed. The linear form of the rainfall term (which resulted from squaring of the square root transformation of the rainfall amount) in 1991 showed a significant but solely negative association with the numbers of deposited ascospores. In 1992, the latter association was not significant (Table 2). The interpretation of the reported findings is that high rainfalls did not induce but rather reduced the dispersal of ascospores. It appears that, for the latter spores to be dispersed, rainfall frequency was more

Fig. 2. Seasonal distribution of (A) *Phaeosphaeria* spp. ascospores and (B) *Stagonospora* spp. pycnidiospores sampled on microscope slides over the years 1990 to 1992.

important than the rainfall amount. In the multiple regression models, combining effects of all weather variables, the effect of the relative air humidity was positively associated with the numbers of airborne ascospores and more strongly pronounced in 1991 than 1992 (Table 2). However, in contrast to the effects of air temperature and the amount of rainfall, an excessively high relative air humidity did not influence the number of ascospores sampled. The latter finding is evidenced by the lack of significance (P value around 0.2) of the squared relative humidity term (Table 2). Therefore, the variable entered neither the 1991 nor the 1992 multiple regression models (Table 2).

The third degree interaction terms of temperature $\times$ sqrt rainfall $\times$ relative air humidity, and the second degree terms of sqrt rainfall $\times$ temperature and sqrt rainfall $\times$ relative air humidity, appeared to be not significant in both years of the study. The prevailing lack of significance (P values ranged from 0.2 to 0.7) of the third and second degree interaction terms between the weather variables points to their very limited influence on ascospore dispersal. In 1992, among the interactions, only sqrt rainfall $\times$ relative humidity was significant, where it had an adverse effect on the amount of airborne ascospores (Table 2).

Finally, after testing individual terms in the models for significance (Table 2), five independent weather variables with significant partial correlation coefficients remained. These entered the multiple regression models and were of key importance for air dispersal of *Phaeosphaeria* spp. ascospores. While the partial correlation coefficients showed the degree of association between individual weather variables and counts of ascospores, the regression coefficients provided a quantitative estimate of the net effects of the weather factors on the numbers of ascospores during each period (Table 2).

In summary, the multiple regression analyses determined the relative importance of each variable which entered a regression model. Ascospore dispersal was significantly influenced individually by each of the weather variables and in one case by their interaction (i.e., sqrt rainfall $\times$ relative air humidity). Although, the relative importance of the weather factors varied, they still could be ranked according to their effect. Thus, air dispersal of *Phaeosphaeria* spp. ascospores was affected most by rainfall, followed by air temperature and relative air humidity. The interaction terms, except for the one mentioned above, did not significantly influence ascospore dispersal (Table 2).

Model validation. The predictive ability and accuracy of the regression models was verified by jackknifing and cross-validation techniques, as well as by examining the models by a substitution of one year's data set with the other. The jackknife estimates of the regression model parameters for each data set differed slightly from the estimates obtained from using all the data or using data of the other sets (Tables 3 and 4). The predictive accuracy of the models developed with 1991 and 1992 data was examined by comparing the prediction variances (S_{cv}^2) and coefficients of determination (R_{cv}^2) from the cross-validation with those from fitting the model when all data were included for both estimation and prediction variances estimation (S_o^2 and R_o^2 , respectively; Tables 3 and 4). The values of overall prediction variance and the overall determination coefficient for 1991 were $S_o^2 = 0.258$ and $R_o^2 = 0.486$, respectively. These were larger than those calculated for 1992 by about one third. Values of overall prediction variance and the overall determination coefficient for 1992 were $S_o^2 = 0.161$ and $R_o^2 = 0.347$, respectively. Values of prediction variances from cross-validation ($S_{cv}^2 = 0.286$ and $S_{cv}^2 = 0.186$ for 1991 and 1992, respectively) differed by the same magnitude between both years (Tables 3 and 4). Differences between the overall and the cross-validation variances were not large and amounted to about 10 and 15% for the respective years of the study. Residual plots between the residuals and the predicted values in both years did not show any relationships. Plots of the predicted against the observed values of ascospores/slide/day were clearly associated, with Pearson $r_{0.000} = 0.697$ and $r_{0.001} = 0.589$ for 1991 and 1992, respectively (Fig. 3A,B). The association between the values observed in 1990 and predicted by the regression models based on data of 1991 and 1992 was Pearson $r_{0.001} = 0.655$ and $r_{0.05} = 0.429$ (Fig. 4).

DISCUSSION

The study showed that ascospore dispersal of *Phaeosphaeria* spp. is not a local phenomenon, i.e., restricted to a single crop field. The data clearly show that the spore inoculum of *Phaeosphaeria* spp. is omnipresent in the air over cereal areas of central Poland during the entire growing season, which for winter and spring cereals is October to early August and April to late August, respectively. The maximum density of inoculum occurs from August through October. These patterns of airborne inoculum distribution are explained by an increasing supply of infected plant material during a growing season. The abundance of ascospores reaches its peak at harvest time. In the months following harvest, plant debris decomposes and the quantity of airborne spores is reduced. Also, adverse climatic conditions of the

Fig. 3. Observed versus predicted numbers of ascospores per microscope slide in (A) 1991 and (B) 1992 from the cross-validation procedure.

late fall and winter in central Poland hamper the formation, liberation, and dissemination of *Phaeosphaeria* spp. spores. Our findings for Poland coincide with findings reported for Germany (29) and New Zealand (18,32). Similarly, ascospores of *Mycosphaerella graminicola* were liberated and dispersed mainly from late summer through spring, with the maximum in autumn (8,34). In contrast, the maximum dispersal of *P. avenaria* f. sp. *avenaria* ascospores was observed over the summer, not during the autumn (9). In the present study, pycnidiospores of *Stagonospora avenae* f. sp. *triticea* and other stagonosporoid fungi were observed on slides, but no attempt was made to determine their percentage.

Pycnidiospores dispersed by wind in water microdroplets were recorded during shorter periods of time, mainly in the late summer (13). This could be explained by the fact that, at this time, other crops were being removed from fields by harvest. In open fields, small droplets of rain can be carried for longer distances (12,36).

Numbers of ascospores were consistently low over all 3 years, even at times of maximum sampling and/or impaction on microscope slides. It was calculated that, on average, the numbers of ascospores sampled per 1m² per day were 242, 205, and 84 in 1990, 1991, and 1992, respectively. The mean value for 3 years was 168 ascospores/m². In contrast, numbers of pycnidiospores sampled per square meter per day were up to 16-fold larger, amounting to 3,326 and 1,111 spores/m²/day for 1991 and 1992, respectively.

It is concluded that the air dispersal of ascospores and pycnidiospores plays a role in the epidemiology of *Phaeosphaeria* (*Stagonospora*) blotches under the climatic conditions of central Poland. The question of which spores are more important in the epidemiology of the diseases is not fully answered by this study. Mittelstädt and Fehrmann (29) expressed an opinion that the importance of airborne ascospores had been underestimated as a potential inoculum source. Verreet (35) still rated the ascospores of little epidemiological importance, though he admitted that they are partly responsible for primary infections of young wheat leaves. Our data indicate that the air dispersal of pycnidiospores is more subject to adverse environmental conditions than is dispersal of ascospores. However, if conditions are conducive for their dispersal, pycnidiospores are dispersed in much larger numbers than ascospores. Pycnidiospores are produced in greater abundance than ascospores and they are the predominant inoculum source. The abundance of pycnidiospores probably also reflects their greater importance in the epidemiology of diseases caused by *Stagonospora* spp. (Figs. 1B and 2A). However, judging by our data, the importance of ascospores as an inoculum source can not be overlooked.

Rapilly et al. (30) observed active release of *P. nodorum* ascospores at temperatures of 0 to 31°C, and noted that temperatures above 25°C were optimal for ascospore discharge. Verreet (35) reported the greatest discharge of ascospores of *P. nodorum* at mean daily temperature between 6 and 15°C, with an optimum of 12 to 15°C. Below 3 and above 15°C, the number of spores was significantly lower. The findings of Verreet (35) closely correspond to results of the present study, which indicated that excessively high or low temperatures reduced the numbers of airborne spores.

Air temperature influences the formation of ascospores, but not their release. Frosts temporarily interrupted the discharge of *Mycosphaerella graminicola* ascospores (8). The same phenomenon was observed in the present study for *Phaeosphaeria* spp. Ascospores, which were dispersed when temperature increased above 0°C following frosty days.

Sanderson and Hampton (32) and Verreet (35) reported that *P. nodorum* ascospores were trapped in large numbers within a few hours after rain and at relative air humidity of 90 to 99%. In the absence of rain, their release occurred during periods when dew usually forms and during periods of ground fog. Our study also revealed that rain was of paramount importance in both years of the study for release and dispersal of *Phaeosphaeria* and *Stagonospora* spores. Brown et al. found that ascospores of *M. graminicola* were always sampled following rain, and frequently when periods of leaf wetness without measurable rain (less than 0.25 mm) were recorded (8). Ascospores of *P. avenaria* f. sp. *avenaria* were ejected from infected oat straw 3 to 12 hours after being placed in moist chambers (9). Hasnain (18) observed that rain increased atmospheric concentration of *Leptosphaeria* spp. and other unidentified ascospores. We found that less than 1 mm of rainfall and high relative humidity (Tables 1 and 2, Fig. 1A-C) were enough to increase the presence of ascospores in the air. Lyon et al. (27) found that precipitation and air humidity were

significantly correlated with concentrations of ascospores in the air, which is in good agreement with findings of the present study. In addition to the above discussed reports, our study showed a limited effect of interactions between the weather variables on spore dispersal of *Phaeosphaeria* spp.. The interaction of sqrt rainfall × relative air humidity exerted an adverse effect on ascospore dispersal, but in only 1 of the 2 years (Table 2).

Results of the present study showed that the splash-borne pycnidiospores required a rainfall event and higher air temperatures than that required for liberation of ascospores. Griffiths and Ao (14) recorded dispersal of *Stagonospora nodorum* spores on all days when rain was falling. *S. nodorum* pycnidiospores were dispersed by splash only when more than 5 to 10 mm of rain fell and air temperature was above 10°C (21,22).

P. nodorum has lower thermal requirements for the teleomorphic stage than the anamorphic stage (15,16). The mating process and production of pseudothecia in vitro was greatest at 10°C. Above 14°C, production of pseudothecia stopped completely. In the present study, minimal air temperatures of about 10°C prevailed from the mid-August until mid October 1991 and during June and September 1992. The most abundant dispersal of ascospores was observed during those periods.

Three major weather variables—rainfall, air temperature, and relative humidity—exerted a significant effect on the presence in the air of *Phaeosphaeria* spp. ascospores. The established relationships showed that the amount of ascospores present in the air was actually a function of the weather variables and remained largely under their control. Air temperature around 13°C was found to be conducive for ascospore dispersal. Overly high or low temperatures reduced numbers of sampled ascospores (Tables 1 and 2). Also, overly high daily rainfall was not necessarily the most favorable for ascospore dispersal. The range of atmospheric conditions stimulating air dispersal of ascospores was much wider than that for pycnidiospores.

Fig. 4. Comparisons predicted by the 1991 and 1992 multiple regression models and observed in 1990 mean weekly counts of *Phaeosphaeria* spp. ascospores settled per microscope slide.

The jackknife and cross-validation techniques (20) proved to be useful to validly estimate parameters and test the performance of models developed on limited data sets. The jackknife-estimated and cross-validated independent weather variables retained in the multiple regression models after significance testing appeared to be reasonably accurate predictors of ascospores deposited on microscope slides. In all cases, the predicted values reflected trends in the observed counts of ascospores (Tables 3 and 4, Figs. 3A and B, 4). Thus, it is concluded that the regression models developed for both periods were of an acceptable predicting capacity and may lay the foundation for a forecasting system for air-dispersed *Phaeosphaeria* spp. ascospores.

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