

## NUMERICAL AND QUALITATIVE ANALYSIS OF ASCOSPORE DISCHARGE OF *Venturia inaequalis* IN CENTRAL POLAND IN RELATION TO WEATHER CONDITIONS

Sylwester Masny<sup>1</sup>  <https://orcid.org/0000-0002-0052-7625>

Paweł Jankowski<sup>2</sup>  <https://orcid.org/0000-0002-3176-8798>

Arne Stensvand<sup>3</sup>  <https://orcid.org/0000-0002-8630-2434>

<sup>1</sup>The National Institute of Horticultural Research, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

<sup>2</sup>Department of Computer Information Systems, Institute of Information Technology, Warsaw University of Life Sciences-SGGW, Nowoursynowska 159, 02-776 Warszawa, Poland

<sup>3</sup>Division of Biotechnology and Plant Health, Norwegian Institute of Bioeconomy Research (NIBIO), Ås 1431, Norway

 [sylwester.masny@inhort.pl](mailto:sylwester.masny@inhort.pl)

### ABSTRACT

The study presents the results of nine years of field observations of ascospore release of *Venturia inaequalis* in the Skierniewice area in central Poland. In total, spores were trapped 221 times during 2005–2008 and 2010–2014. Of these, 142 episodes lasted for less than 8 hours, 69 lasted from 8 to 29 hours, and 10 episodes lasted from 30 to 93 hours. Spore releases started in spring from 25 March to 28 April and ended from 27 May to 17 June, and the season for ascospore release lasted from 43 to 76 days, with an average of 58 days. During 139 ascospore releases, less than 1 000 spores per cubic meter of air were collected and during 25 discharges more than 10 000 spores were trapped. Releases of ascospores were highly related to rainfall and daylight. Two-thirds (67%) of the spores were trapped during rain. Only 12% of the discharges occurred without any registered rain, accounting for 7% of all trapped spores. Nearly three fourth (73%) of all ascospore release hours occurred in daylight, and 91% of the spores were trapped in daytime. Rainy nights with constant leaf wetness were observed, during which no spore releases occurred despite the rainfall. Ascospore releases were also less prominent at the beginning and end of the season and after weak rains. Rain was most effective as the trigger of discharges at temperature between 5 and 13 °C and when global radiation coinciding with rainfall was below 700 W/m<sup>2</sup>. In conclusion, the study confirms the dominant role of daytime rainfall in the release of ascospores by *Venturia inaequalis*.

**Keywords:** apple scab, computer calculations, *Malus × domestica*

### INTRODUCTION

The most important disease in apple production in Poland, similar to that in temperate regions throughout the world, is apple scab caused by the fungal pathogen *Venturia inaequalis* (Cooke) G. Winter [MacHardy 1996]. The control of apple scab is responsible for 70% of the pesticide use in apple production [Creemers and Laer 2006].

The localization of the investigation took place close to the Grójec area, which is one of the largest regions of apple production in Poland, and was carried out in 2005–2008 and 2010–2014. In our previous studies, we focused on modelling the influence of moisture on maturation rate of ascospores of *V. inaequalis* [Jankowski and Masny 2019, 2020]. Pseudothecial development and severity of apple scab in the same area were discussed by Mieszka [2015].

The predominant primary inoculum of *V. inaequalis* in spring is ascospores released from pseudothecia in leaf litter on the ground. Numerous reports have shown that rainfall is the primary reason for ascospore re-

lease in *V. inaequalis*, including Brook [1969], MacHardy and Gadoury [1986], Rossi et al. [2001], and Alt and Kollar [2010].

Another major factor affecting the quantity of ascospore discharge in *V. inaequalis* is global radiation. Again, the importance of daylight to trigger high releases of ascospores in *V. inaequalis* has been reported by many authors, such as Brook [1969], MacHardy and Gadoury [1986], Warner and Braun [1992], Gadoury et al. [1998], Rossi et al. [2001], and Villalta et al. [2002].

Another important weather parameter that influences the length of ascospore release seasons is periods of dryness. As shown, for example, by Stensvand et al. [2005], long, dry periods during the period of ascospore maturation will extend the season of ascospore release.

In this work, we aim to present a numerical and qualitative description of the influence of weather conditions on the timing of ascospore discharges of *V. inaequalis* in the Grójec area, Poland. We want to verify the observations made by other authors under climatic conditions in central Poland. Nine seasons of field observations of ascospore release by *V. inaequalis* were used to analyse the relationship between spore discharge and weather factors.

## MATERIALS AND METHODS

### The experimental site

Spore trapping was carried out in an experimental orchard at The National Institute of Horticultural Research (InHort) in Skierniewice (N 51°55'; E 20°6'), located in the Grójec area of central Poland. The data analysed in the study were collected during 2005–2008 and 2010–2014. Analysis of weather factors in each year suggested incorrect measurements of the leaf wetness sensor in the season 2009. Therefore, data from 2009 were excluded from the analysis.

### Monitoring of weather conditions

Weather monitoring was conducted in the orchard. Air temperature (°C), precipitation (mm, with 0.2 mm as the lowest amount measured), relative humidity (RH, %), occurrence of leaf wetness (LW), and global radiation ( $W/m^2$ ) were recorded continuously from January to June by an automated weather station (model Metos-Compact, Pessl Instruments, Weiz, Austria). Leaf wetness was measured at a height of 20 cm above the ground. The other weather parameters were measured at a height of 2 m. Data were collected at intervals of 12 minutes and averaged at hourly intervals. Precipitation and RH sensors were calibrated each year. All hours of the day presented in the manuscript were adjusted to Central European Time (CET). CET was used consistently during the whole year, so Central European Summer Time (CEST) was never used.

Throughout this article, hours with daylight were defined as the hours with observed global radiation and the remaining hours as night hours.

Degree-day (DD) accumulation (base = 0 °C) was computed for each year from the start to the end of the spore trapping seasons. The adjusted DD accumulation ceased after four consecutive days without measurable rain. Accumulation then resumed when a rain event terminated the “dry” interval. Following the proposal of Stensvand et al. [2005], “dry” years were defined as years in which the difference between unadjusted versus adjusted DD accumulation exceeded 200.

### Ascospore trapping

A Burkard 7-day recording volumetric spore trap (Burkard Manufacturing Co Ltd., Rickmansworth, Hertfordshire, UK) was used to monitor the release of ascospores from the leaves of *Malus × domestica* (Suckow) Borkh. cv. McIntosh. Approximately 2 m<sup>2</sup> of the ground under the spore trap was covered with apple leaves heavily infected by *V. inaequalis* in the previous season. The leaves were gently arranged in autumn after most of the leaves had fallen from the trees. They were covered with plastic netting to prevent them from being moved by the wind. The orchard area surrounding the spore trap (0.2 hectares) was not treated with fungicides during the 9-year period of spore trapping. The spore trap was installed in the centre of this area and was adjusted to sample air 1 m above the ground at a rate of 0.6 m<sup>3</sup> h<sup>-1</sup>. The tape from the Burkard spore trap (with a width of 19 mm and a length of 336 mm) was changed weekly. The weekly segments of the tape were cut into seven 48 mm long segments, representing the length covering a period of one day of sampling. The number of ascospores of *V. inaequalis* deposited on each 2 mm width of the tape corresponding to 1-hour sampling, was counted using a microscope (200× magnification). Ascospore counts were converted into counts per cubic meter volume of sampled air per hour.

The end of each discharge period was determined as the hour after which no ascospores were trapped for at least four hours. Therefore, if an interval without trapped spores between two periods with spore release lasted less than four hours, this was considered one discharge period.

## Statistical analysis

Data analyses were performed using GNU Octave version 7.3.0, and R program version 4.2.2 with the R Studio version 2022.12.0.

The Spearman correlation was used to analyse the relation between diurnal cycles of precipitation, global radiation, RH, and temperature with start of spore discharges and abundance of trapped ascospores.

The exact Fisher's test was performed to analyse the relationship between observation of an ascospore discharge during a rainy hour and rainfall amount, LW occurrence, RH, temperature, and global radiation. The same test was used to examine the differences between the characteristics of ascospore releases caused by rainfall and releases not directly related to rain.

The Kruskal-Wallis analysis was applied to compare the distribution of temperature and RH in selected groups of hours of the observed seasons. The Dunn's post-hoc test was used to identify their homogenous groups. The proportions of hours with wet leaves in these sets of hours were compared using the pairwise test of proportions with the Benjamini and Hochberg adjustment method.

## RESULTS

### Length and abundance of the observed ascospore discharges

In 2005–2008 and 2010–2014, the seasons for ascospore release of *V. inaequalis* in Skierniewice started between 25 March and 28 April and ended between 27 May and 17 June (Table 1). The duration of ascospore trapping ranged from 43 to 76 days, with an average of 58 days. Accumulation of DDs and adjusted DDs from start to the end of each spore trapping season varied from 586 to 886 and from 433 to 612, respectively. Seasons that can be considered “dry”, i.e., years with a difference between unadjusted and adjusted accumulation of 200 DDs or more, were 2005, 2006, 2008, and 2012. There were 221 periods when ascospores were trapped during the nine seasons, lasting a total of 2054 hours (16.3% of all hours), with 1797 hours (14.3%) with trapped ascospores. The majority of the discharge periods (142) lasted less than 8 hours; 55 lasted between 9 and 17 hours; and 24 over 17 hours. The 10 longest release periods lasted 30 to 93 hours. When adjusted for volume of air, the total seasonal number of ascospores trapped varied from less than 10 000 to approx. 550 000 (Table 1). During 139 release periods, less than 1 000 spores were trapped; during 57 periods between 1 000 and 10 000 were trapped; and over 10 000 spores were trapped during 25 discharge periods. The highest trapping of ascospores was approx. 213 000 over a period of 30 hours in 2013.

**Table 1.** Characteristics of the *Venturia inaequalis* ascospore seasons during nine years (2005–2008 and 2010–2014) in an experimental orchard in Skierniewice, Poland. The dates (month–day) of the first and last trapping of ascospores, season lengths (in days and hours), degree-days, the total number of trapped ascospores, and percentage of hours of discharge events and hours with trapped spores

Year	Date of		No. of		Degree-days		Ascospores trapped	Percentage of hours	
	start	end	days	hours	unadjusted <sup>1</sup>	adjusted <sup>1</sup>		of discharges <sup>2</sup>	with trapped spores <sup>2</sup>
2005	04–28	06–17	52	1247	667	433	48340	15.5	13.8
2006	04–16	06–17	63	1511	812	590	211901	12.2	11.3
2007	04–09	06–02	55	1327	692	543	9291	12.9	9.6
2008	03–29	06–13	77	1859	886	457	118365	13.2	11.8
2010	04–02	05–27	56	1349	586	575	328592	26.4	22.1
2011	04–04	05–28	55	1324	631	477	86455	18.5	16.0
2012	04–13	06–14	63	1521	871	593	183832	12.4	11.4
2013	04–19	06–01	44	1062	598	467	548080	25.0	23.1
2014	03–25	05–29	66	1591	730	612	40609	12.8	11.2

<sup>1</sup> Degree-days (base = 0 °C) accumulated from start to end of each spore trapping season. The adjusted degree-day accumulation ceased after four consecutive days without measurable rain. Accumulation then resumed when a rain event terminated the dry interval.

<sup>2</sup> Any interval of less than four hours without trapped spores between two hours with trapped spores was considered part of a discharge event. The end of each discharge period was determined as the hour after which no ascospores were trapped for at least four hours.

### Weather during the ascospore release season

Details about the weather characteristics in each season are given in Table 2. Over the nine seasons, 972 hours (7.6% of all hours) with rainfall were observed at the location of the experiment. During 34.3% of the rainy hours, the precipitation equalled 0.2 mm. For 38.3% and 22.1% of the rainy hours, precipitation was 0.2 to  $\leq 1$  mm and  $>1$  to 3 mm, respectively. Rainfalls higher than 3 mm were observed in only 5.3% of the events. Leaf wetness was observed during 22.5% of the analysed hours. About two-thirds (64%) of the rainy hours and 68% of the amount of precipitation were registered during daytime. The share of the hours with daylight was 66.5%.

The lowest and highest temperatures during the nine seasons were  $-4.7$  °C and  $31.8$  °C, with a mean of  $12.2$  °C. The lowest RH during the whole period of investigation was 22.8%, with a mean of 79.1%, and the fraction of the hours with  $RH \geq 99\%$  was 23%.

**Table 2.** Weather characteristics for all hours of the *Venturia inaequalis* ascospore seasons, starting from the first and ending with the last trapping of ascospores, during nine years (2005–2008 and 2010–2014) in an experimental orchard in Skierniewice, Poland. Percentage of hours with observed rainfall ( $\geq 0.2$  mm), recorded leaf wetness (LW), and daylight. The extremes (minimum and maximum) of temperature (°C) and minimal percentage relative humidity (RH)

Year	Percentage of hours			Temperature [°C]		RH [%]
	rainy	with LW	with daylight	minimum	maximum	minimum
2005	6.2	19.0	69.2	0.6	31.8	39.4
2006	5.8	13.2	68.2	2.8	26.8	26.2
2007	7.7	21.8	66.4	-4.1	30.6	26.0
2008	5.5	15.9	65.6	-2.2	27.7	34.2
2010	10.0	29.9	64.7	-2.2	25.2	22.8
2011	6.3	20.3	65.4	-1.4	26.4	25.4
2012	8.2	18.0	68.4	-1.4	29.4	23.4
2013	12.1	30.6	67.0	0.9	26.5	30.4
2014	8.2	21.1	64.6	-4.7	27.6	28.2
Total	7.6	22.6	66.5	-4.7	31.8	22.8

**Table 3.** Weather characteristics for hours when ascospores of *Venturia inaequalis* were trapped during nine years (2005–2008 and 2010–2014) in an experimental orchard in Skierniewice, Poland. Percentage of hours with observed rainfall ( $\geq 0.2$  mm), recorded leaf wetness (LW), and daylight. The extremes (minimum and maximum) of temperature (°C) and minimal percentage relative humidity (RH)

Year	Percentage of hours			Temperature [°C]		RH [%]
	rainy	with LW	with daylight	minimum	maximum	minimum
2005	19.7	60.5	76.7	3.7	23.6	58.8
2006	28.1	59.8	68.6	6.6	23.2	51.8
2007	38.0	69.8	76.0	4.4	30.6	45.0
2008	31.7	75.9	69.5	0.75	19.0	69.8
2010	29.2	69.9	67.1	1.7	20.6	45.2
2011	28.2	61.9	72.2	-0.52	22.5	38.2
2012	46.3	76.4	79.8	-0.09	22.8	50.6
2013	32.7	72.9	74.1	5.0	23.1	54.0
2014	45.1	82.8	81.9	1.4	19.2	45.2
Total	32.7	74.7	73.3	-0.52	30.6	38.2

The weather characteristics during periods of ascospore release in each season are given in Table 3. One-third (33%) of the hours with trapped ascospores of *V. inaequalis* occurred during rainfall, leading to 42% of the total number of spores trapped. The rainy hours during which ascospores were trapped accounted for 61% of all rainy hours during the nine seasons. Over one-half (51%) of the discharge hours, and 67% of spores were observed from 1 hour before registered rainfall ( $<0.2$  mm) to 1 hour after the rainfall. Moreover, nearly two in five (38%)

observed spore release periods started during a rainfall. The share of discharges that started from 1 hour before to 1 hour after rainfall was 56%, and the share of ascospores fell rapidly with the increasing distance in time from recent rainfall. Only 10.5% of the ascospores were observed 4 to 6 hours after rain and 4.5% after a dry period longer than six hours. The 49 (12% of all) discharges without any rainfall observed accounted for about 7% of all trapped spores. Of all ascospore release hours, 73.3% occurred in daylight, and 91% of the spores were trapped during those hours.

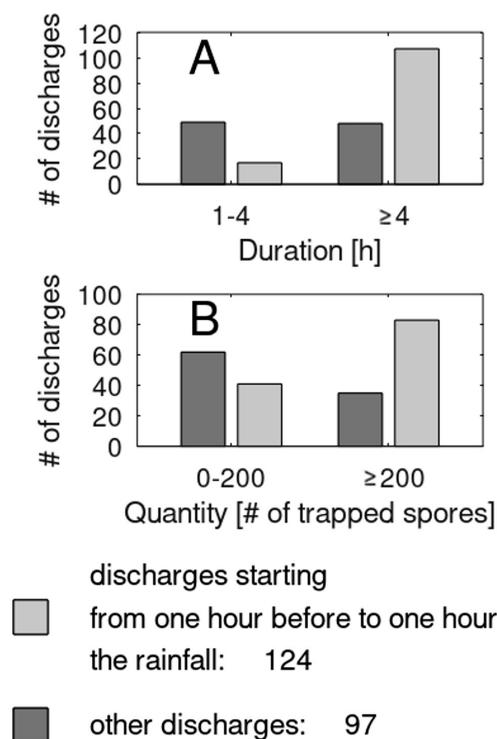
The absolute minimum and maximum temperatures of all periods during which ascospores were trapped were  $-0.5$  and  $30.6$  °C, respectively, with a mean of  $10.4$  °C. The lowest RH during the release periods was 38.2, with a mean of 91.6%, and 44.2% of the hours had  $RH \geq 99\%$ .

### Start of ascospore release either related or not related to rainfall

The start of the observed periods of spore release was divided in two groups depending on the distance in hours from rainfall. The first group consisted of 124 (56% of all) discharges in which the first ascospores were trapped from 1 hour before to 1 hour after the closest rainfall. The second group contained the remaining 97 discharges.

Trapping of ascospores from the first group lasted most of the time for 4 hours or more, and the majority of the episodes contained more than 200 spores (Figure 1). These discharges corresponded to 63% of the length of all spore release periods and to 60% of the total number of ascospores trapped. Approximately the same number of spore release periods from the second group lasted from 1 to 4 hours (49 discharges) and for more than 4 hours (48 discharges). During most of these episodes of spore release less than 200 spores were trapped. According to the Fisher's exact test, differences between the two groups of discharges were significant for both length and abundance ( $p < 0.0001$ ).

**Figure 1.** Histograms comparing duration of ascospore trapping (A) and numbers of trapped ascospores (B) of *Venturia inaequalis* during discharges in which the first ascospores were trapped from 1 hour before to 1 hour after the closest recorded rainfall (light grey) and the remaining ones (dark grey)



### Influence of weather on occurrence of trapped ascospores during rainfall

The comparison of weather conditions during the rainy hours with and without trapped spores is presented in Figure 2. Each observed variable was split into ranges with a higher number of rainy hours either with or without ascospores.

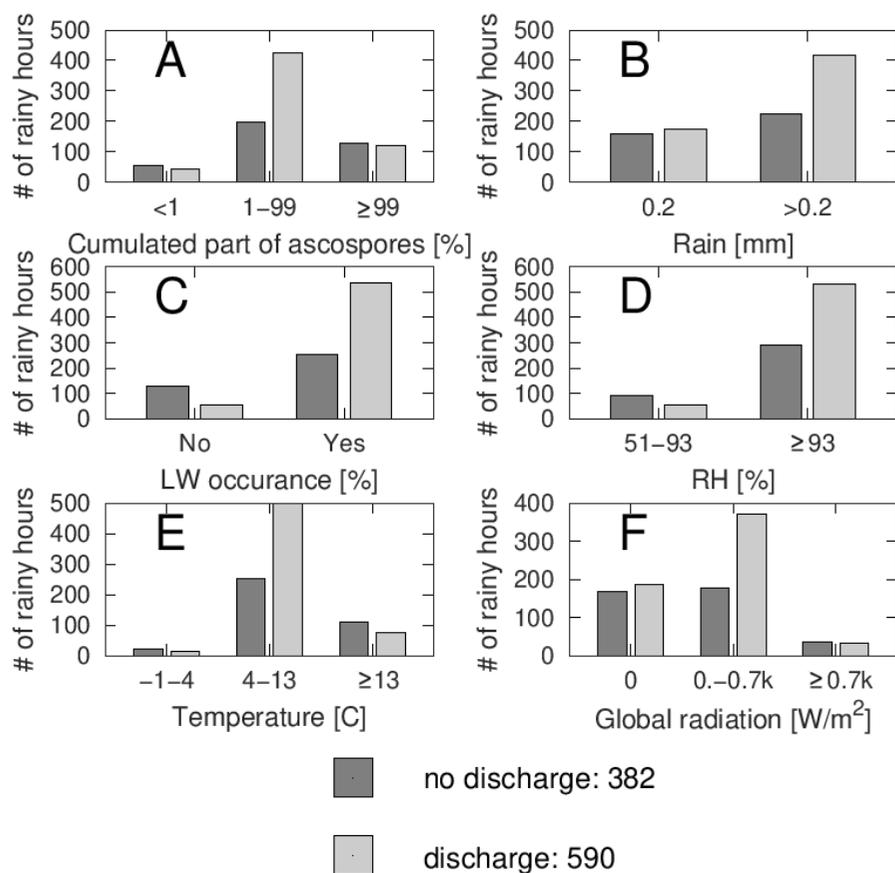
The  $p$ -value of Fisher's exact test for independence was computed for each variable. The immediate ascospore releases were least frequently caused by precipitation in the beginning and end of the seasons, when either less

than 1% or more than 99% of the seasonal spores had been trapped, respectively (Figure 2A). The weather conditions during a rainy hour, less favourable for immediate ascospore discharges, were as follows:

- i) rain equal to 0.2 mm, with a relatively higher number of hours with vs. without trapped ascospores (Figure 2B);
- ii) LW was recorded (Figure 2C);
- iii) RH was below 93% (Figure 2D);
- iv) temperature was below 4 °C or above 13 °C (Figure 2E);
- v) during night when there was no global radiation, or
- vi) when global radiation coinciding with a rainfall was above 700 W/m<sup>2</sup> (Figure 2F).

All the observed relations were statistically significant, at  $p < 0.001$ . No statistically significant impact of wind speed on the ascospore discharge during a rain was found.

**Figure 2.** Histograms comparing conditions during the rainy hours without (dark grey) and with (light grey) trapped ascospores of *Venturia inaequalis*. Number of hours in the two groups versus: (A) percentage of cumulated seasonal spores (%), (B) the amount of precipitation (mm), (C) occurrence of leaf wetness (LW), (D) relative humidity (% RH), (E) temperature (°C), (F) global radiation (W/m<sup>2</sup>)

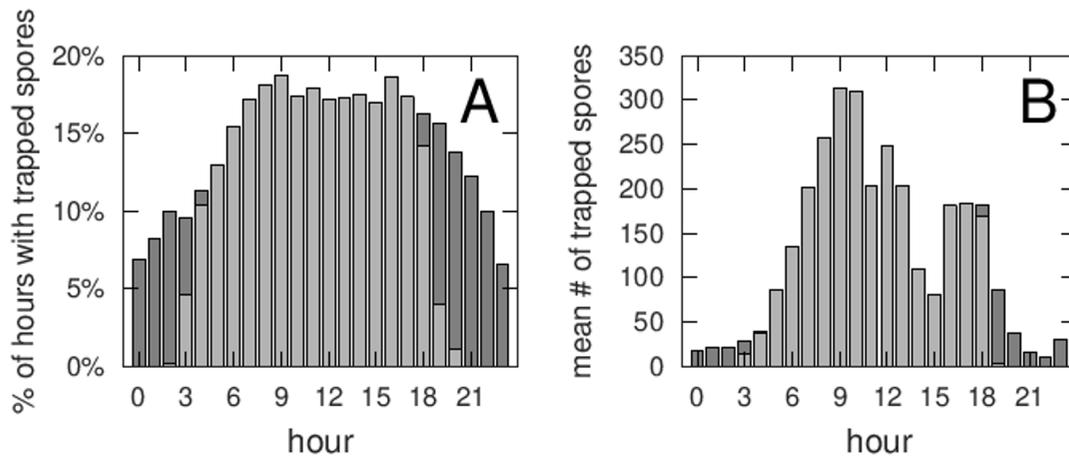


### Relation between diurnal cycles of ascospore abundance and diurnal weather cycles

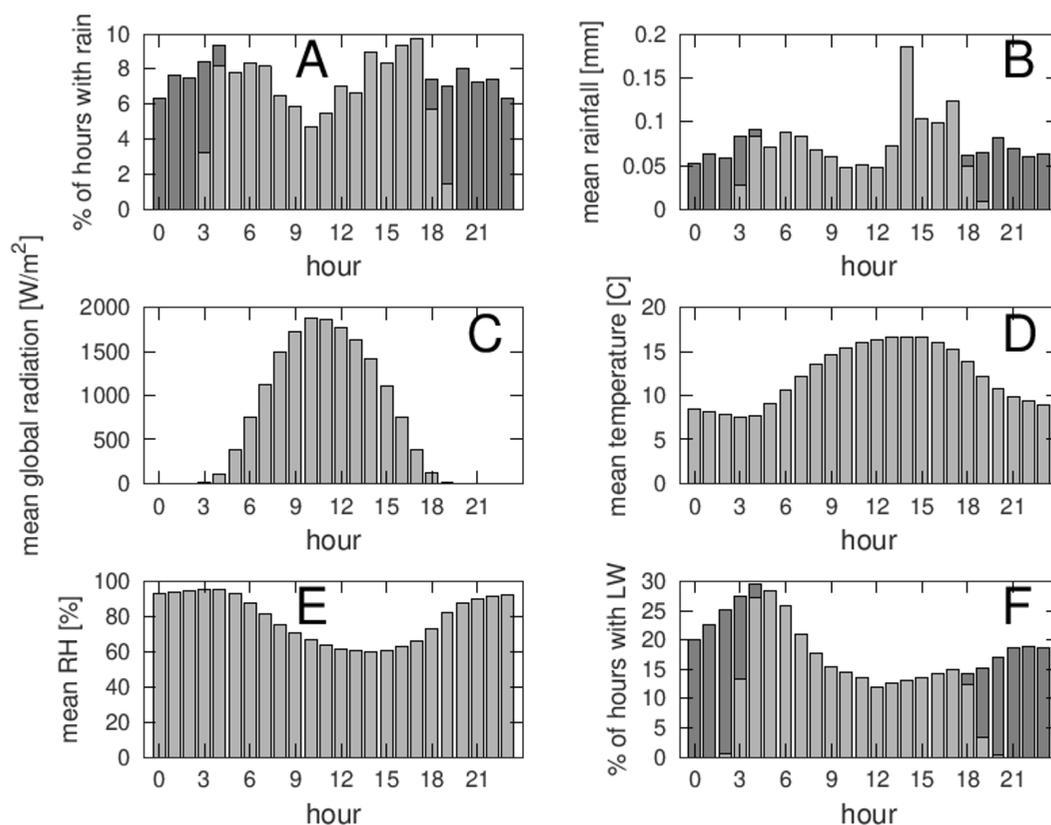
The share of hours with trapped ascospores increased from midnight, attaining a flat plateau between 7 and 18, when it started to decrease until 23 (Figure 3A). During the period of maximum spore trapping, there were two separate maxima around 8–12 and 16–18 (Figure 3B).

The diurnal distribution of the observed weather factors formed two groups. The first one was related to precipitation. The diurnal cycle of the number of rainy hours during the observed seasons showed two broad peaks (Figure 4A). The first peak was in the morning, with the highest values at the verge of night and day around 3–4. The second one occurred in the afternoon, with the highest number of rainy hours around 14–17. The distribution of the amount of rainfall showed the same two peaks with the maximum rate at 14, due to the largest single rainfall of 42.8 mm observed in the 9-year study (Figure 4B). The least amount of rain was observed around midnight and at 9–11.

**Figure 3.** Diurnal cycles averaged from nine seasons (2005–2008 and 2010–2014) of ascospore trapping of *Venturia inaequalis* at Skierniewice, Poland: (A) the percentage of hours with trapped spores, and (B) the mean number of spores. The data is divided into daytime (light grey) and nighttime (dark grey) contributions



**Figure 4.** Diurnal cycles averaged from nine seasons (2005–2008 and 2010–2014) of ascospore trapping of *Venturia inaequalis* at Skierniewice, Poland: (A) percentage of hours with rain, (B) mean rainfall (mm), (C) mean global radiation ( $W/m^2$ ), (D) mean temperature ( $^{\circ}C$ ), (E) mean relative humidity (RH, %), and (F) percentage of hours with observed leaf wetness (LW). The data presented in plots (A), (B), and (F) is divided into daytime (light grey) and nighttime (dark grey) contributions



The diurnal cycles of LW, RH, temperature, and global radiation formed a second group. The increase of the average global radiation started from 03 to its peak at 10–11 (Figure 4C). The mean temperatures showed a more shallow but broader peak than the average global radiation (Figure 4D), and that maximum corresponds with the lowest RH in the diurnal cycle (Figure 4E). The diurnal cycle of the number of hours with wet leaves (Figure 4F) was closely related to the cycle of RH. The exception is the strong morning peak in number of hours with wet leaves around 3–4, which corresponds to the morning peak of rain intensity.

The diurnal distribution of weather factors were highly correlated within each group (Figure 5). The diurnal cycles of the hours of ascospore release and the average number of observed ascospores were significantly correlated only with the second group of weather factors: global radiation, temperature, relative humidity, and LW occurrence (Figure 5). The observed correlations were positive for global radiation and temperature and negative for RH and LW. All correlations were statistically significant at  $p < 0.001$  with two exceptions, diurnal cycle of the hours with LW occurrence was correlated at  $p < 0.01$  with cycles of average global radiation and with average number of trapped ascospores.

### Periods of rainfall with no ascospores observed

Three groups of rainy hours during which no ascospores were trapped were selected after the detailed analysis.

The first group contained rainfalls which occurred directly before the start of ascospore discharges. In each case, the first rain started a period of constant leaf wetness ending up with ascospore discharge. We observed 71 such periods, varying from 1 to 10 hours, but only 17 periods were longer than 3 hours. The number of rainy hours which occurred during each period varied from 1 to 8. In the majority (41) of cases only one rainy hour was observed, and only in 9 cases their number was greater than 3 hours. In total, approximately 14% of all rainy hours occurred during these periods.

**Figure 5.** Spearman correlations between the diurnal cycles of intensity of spore trapping (Figure 2) and main weather factors (Fig. 3) averaged from nine seasons (2005–2008 and 2010–2014) of ascospore trapping of *Venturia inaequalis* at Skierniewice, Poland: percentage of hours with rain, mean rainfall, percentage of hours with observed leaf wetness (LW), mean relative humidity (RH), mean temperature, mean global radiation, percentage of hours with trapped spores, and mean number of trapped spores. NS stands for ‘non-significant’

	% of hours with rain						
mean rainfall	0.86	mean rainfall					
% of hours with LW	NS	NS	% of hours with LW				
mean RH	NS	NS	0.92	mean RH			
mean temperature	NS	NS	-0.91	-1.00	mean temperature		
mean global radiation	NS	NS	-0.58	-0.75	0.76	mean global radiation	
% of hours with trapped spores	NS	NS	-0.65	-0.82	0.83	0.86	% of hours with trapped spores
mean # of trapped spores	NS	NS	-0.54	-0.69	0.70	0.93	0.87

The second group contained rainfalls which occurred during the ascospore-free periods separating two spore discharges by more than 3 hours but less than 16 hours. We observed 23 such intervals containing about 9% of all rainy hours. All of them were characterized by persistent leaf wetness. All but one started shortly before or during night and ended during night or in the early morning. During 14 periods the intensity of precipitation was at least 3 rainy hours. There were three very long intervals, lasting from 10 to 15 hours, with high intensity of precipitation for 10 to 12 hours. Similarly, 13 ascospore-free nightly periods between two discharges with constant leaf wetness but no precipitation were observed.

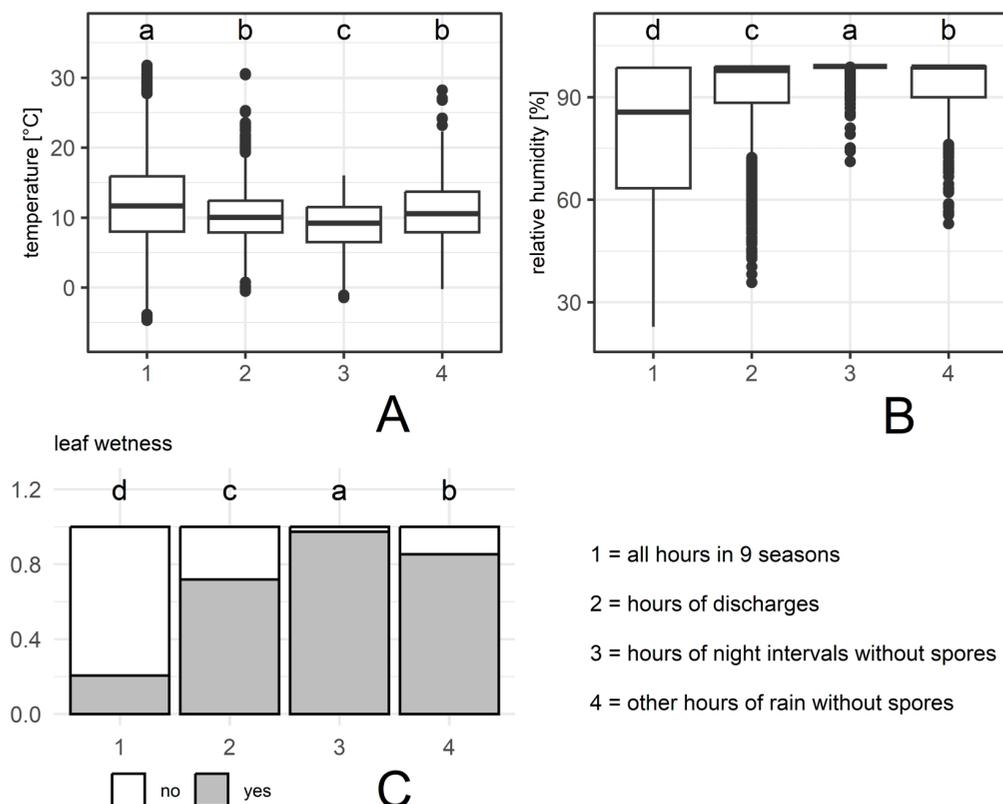
The remaining 75 hours of rainfalls (about 8% of all rainy hours) without trapped spores occurred either shortly after the ascospore discharge when leaves were still wet or when leaves had already dried out. In the latter case, on single occasions the rains were observed a few hours after an ascospore discharge but usually after ten or more hours and over ten hours since a next discharge. In 56 cases they lasted for only one hour. During 53 of the 75 hours only 0.2 mm was recorded.

The distribution of temperature (Figure 6A), RH (Figure 6B), and proportion of hours with observed LW (Figure 6C) was compared within the groups of:

- i) all hours of the observed seasons,
- ii) hours of discharges,
- iii) hours of the 36 night intervals without trapped ascospores, and
- iv) the remaining 75 hours of precipitation without trapped spores.

In case of all three weather factors, the Kruskal-Wallis analysis showed significant differences between the groups of hours ( $p < 0.001$ ). The 36 night intervals were coldest, with highest average RH (97.9%), highest proportion of hours with wet leaves (97.4%), and the fraction of hours with RH  $\geq 99\%$  around 81%.

**Figure 6.** Distributions of temperature (A) and relative humidity (B), and proportions of hours with observed leaf wetness (C) within the groups of: all hours of the observed seasons, hours of discharges, hours of night intervals without trapped spores, and the remaining hours of rainfall without trapped spores. The distributions of temperature and relative humidity were compared with the Kruskal-Wallis analysis and their homogenous groups, denoted with letters, were established according to the Dunn's post-hoc test. The proportions of hours with wet leaves in these sets of hours were compared using the pairwise test of proportions with the Benjamini and Hochberg adjustment method. All differences were significant at  $p < 0.001$



### Supplementary material in Additional file 1

Figure S1 shows the comparison of the dates of the ascospore release seasons and average climate conditions in central Poland with similar data from three European areas where spore trapping of *V. inaequalis* had taken place. Figures S2 to S30 present selected fragments of the analysed nine seasons containing most of the significant ascospore release periods.

## DISCUSSION

In the present work, we analysed nine years of ascospore release of *V. inaequalis* in central Poland. The discharge seasons started from late-March to late-April and ended from late-May to mid-June. They were significantly delayed compared to regions of Europe characterized by a climate with higher temperatures and longer periods of sunshine, such as Avignon, France or Passo Segni, Italy (Additional file 1: Figure S1). In Avignon, the spore trapping (years 1990–1995, 1997–2001 and 2003) started from 23 February to 8 April and ended from 5 May to 6 June [Roubal and Nicot 2016]. In Passo Segni, the spore releases (years 1991–1996) started from 16 March to 2 April and ended from 17 April to 12 June [Rossi et al. 1999]. On the other hand, in the colder climate of Ås, Norway (1990–1995, 1997–2001, and 2003), the first ascospores of *V. inaequalis* were on average observed later than in Skierniewice, from 2 April to 30 April, and 95% of the spore trapping took place from 29 April to 7 July [Stensvand et al. 2005]. This important difference implicates the impact of climate on beginning and end of the seasons for ascospore release of *V. inaequalis*. On the other hand, our results showed that as in the other regions mentioned above, the major factors influencing the timing and intensity of spore releases in central Poland are rainfall and daylight. The presented detailed analysis of nine spore release seasons may be useful in assessing possible changes in dates of ascospore releases with expected climate changes in central Poland. Such changes will likely lead to higher temperatures and change of rainfall pattern in spring and summer [Ghazi et al. 2023], thus influencing the two key factors in spore release, and therefore, in apple scab development.

Numerous studies have reported that rain is the most important factor affecting ascospore release in *V. inaequalis*, and a study by Rossi et al. [2001] indicated that rainfall is the sole factor leading to onset of ascospore discharge of the fungus. According to Alt and Kollar [2010], raindrops are necessary to induce ascospore discharge due to the pressure they exert on the fruiting bodies. Our study also shows that precipitation and spore discharges are highly correlated. In general, the discharges triggered by rainfall were the longest and most abundant ones. Only about one in ten of discharges were completely rainless, and these releases accounted for less than 10% of all trapped spores. Moreover, ascospores were trapped during more than half of the rainy hours during the ascospore release seasons. About half of the discharge hours occurred and two-thirds of the airborne spores were trapped in periods from one hour before to one hour after measured rainfall. In the analysis, it was assumed that the effect of a rainfall on ascospore discharges may be delayed by one hour, similar to the average 1.1 hour delay observed in northern Italy [Rossi et al. 2001]. We also assumed that the observed rain could be preceded by precipitation of an intensity below the sensitivity of the weather station. Moreover, the microscope tape can be slightly skewed in relation to time, thus leading to incorrect timing of assumed ascospore trapping. Hence, we considered the period from 1 hour before to 1 hour after a rainfall as the period of its direct impact on a spore release.

Nevertheless, not all rainfalls led to immediate onset of ascospore discharges. Firstly, spores were less often trapped during rainy hours in the early and late parts of the seasons of ascospore release. This is most likely because of lower numbers of mature ascospores ready for release at that time. The above observation is in agreement with the model of ascospore maturation by Rossi et al. [2007], where the period of low risk of discharges and the period of no risk of discharges were introduced in the model in the beginning and end of a season, respectively.

Secondly, the ability of rain to induce a discharge is affected by the intensity of the rain. In the present investigation, the probability of an immediate release decreased when rainfall intensity was at the detection limit of 0.2 mm. The relation between rain intensity and spore releases explains the relation of the latter with leaf wetness, which usually occurs following rainfall.

Thirdly, rainfalls less frequently led to ascospore release if there was no global radiation (during night) measured or when global radiation was above 700 W/m<sup>2</sup>. Similarly, low or high air temperatures were not conducive to ascospore discharges. Although ascospores were trapped at temperatures varying from about 0 °C to more than 25 °C, more than half of the rainfalls did not result in ascospore releases if temperatures were below 4 °C or above 13 °C. As stated by Stensvand et al. [1997], temperatures of 2 °C or less nearly stop the process of ascospore dis-

charge. On the other hand, high temperatures lead to faster drying of the leaves after rain and therefore decrease the probability of ascospore discharge.

Finally, ascospore trapping seasons in 2006, 2008, and 2012, which were described as “dry” seasons (difference between unadjusted and adjusted accumulation of DDs higher than 200), were among the four longest seasons. This confirms that in central Poland the long, dry periods during ascospore maturation extend the season of ascospore release [Stensvand et al. 2005]. The season in 2005, which was also described as “dry”, was much shorter due to its exceptionally late start.

Daylight was the second factor with a widely recognized importance for spore discharge. Numerous authors have reported the suppression of ascospore discharges at night observed in both field [Brook 1966, 1969, MacHardy and Gadoury 1986, Warner and Braun 1992, Rossi et al. 2001, Villalta et al. 2002] and laboratory [Gadoury et al. 1998] studies. The same phenomenon was observed in the present investigation. More than 90% of the ascospores were trapped during daytime, while the share of hours with daylight was 65%. These results agree with other reports in terms of the proportion of ascospores trapped in the dark, although the numbers reported in different studies have varied. While around 9% of the spores in Skierniewice were trapped during the night, Brook [1966] in New Zealand observed only 0.51%, MacHardy and Gadoury [1986] in New Hampshire in USA below 5%, Rossi et al. [2001] in Italy around 7%, and Villalta et al. [2002] in Victoria in Australia about 18% of ascospores of *V. inaequalis* trapped during darkness.

The ascospore release in central Poland was not only suppressed by the lack of daylight but in general its intensity depended on the intensity of global radiation. When analysing the diurnal cycles of average ascospore discharge frequency and intensity and the cycles of main weather factors, the highest correlation (0.93) was found between the cycles of average global radiation and average number of trapped spores. The latter was correlated with all main weather factors: LW, RH, air temperature, and global radiation, which are strongly related to each other. Most importantly, the diurnal cycle of spore intensity was significantly unrelated to the diurnal cycle of precipitation. How is it possible if, as discussed above, rainfall is so crucial for the onset of ascospore releases?

The answer lies mainly in the role of light and its interaction with the rain [Brook 1969, Gadoury et al. 1998]. Firstly, as already discussed, high global radiation coinciding with rainfall is not conducive for ascospore release, because of the quick drying of leaves. Secondly, during our field experiments we observed night periods between discharges with rainfalls that did not trigger ascospore releases. These periods were characterized by high air humidity and constantly wet leaves that could not dry due to lack of daylight. We assume that fog deposition or dew formation were the causes of leaf wetness. Similar intervals with wet leaves but without noticeable precipitation were also observed between discharges. Although Stensvand et al. [1998] found that dew can cause significant ascospore releases, others [Brook 1969, MacHardy and Gadoury 1986] observed that only rarely small amounts of airborne ascospores were trapped during periods of dew. Rossi et al. [2001] found that nightly rainfalls followed by heavy dew deposition that persisted some hours after sunrise suppressed spore discharges. According to Brook [1969], in the case of dew, the water deposit increases too much and ascospores are discharged into the water and not into the air.

The above issue explains why two maxima in the diurnal cycle of rainy hours lead to different size maxima in the cycle of trapped spores. The smaller afternoon maximum of airborne ascospores, around 16–17 hour, follows the maximum of rainfall intensity that starts around 14 hour. As the daylight soon fades after 17 hour, the spore discharges are suppressed. The more significant morning peak of ascospore intensity, around 8–12 hour, follows the stronger maximum rainfall intensity, around 3–7 hour. This peak corresponds to the most intense radiation period, and the ascospore discharges are not limited by lack of daylight.

### **Study limitations and future research**

A significant study limitation may be that the study only took place in a single localization and with only one apple cultivar. That, and the ongoing climate change and constant modification of the *V. inaequalis* genotype due to its high genetic variation, imply that the relationship between the *V. inaequalis* ascospore releases and weather may have to be analysed more often and, if possible, should be performed based on data originating from various localizations and apple cultivars.

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