

Comparison of the actual release dates of ascospores of the fungus *Venturia inaequalis* with those predicted by selected simulation models in an apple orchard in central Poland

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Abstract: The research was conducted in 2014–2017 in a multi-cultivar apple orchard in the Experimental Orchard of the National Institute of Horticultural Research (IO-PIB) in Dąbrowice near Skierniewice. To determine the actual *Venturia inaequalis* ascospores release dates, the Burkard spore trap installed in a plot of the McIntosh cv. that was not protected against apple scab was used. Monitoring of ascospore releases was carried out annually, starting from the appearance of numerous colouring (maturing) ascospores in the pseudothecia (usually in the second decade of March) and ending at the second half of June, usually about two weeks after the last release of these spores. The sums of ascospores detected on a given day and their proportion in all ascospores recorded during primary infections were calculated. The obtained results formed the basis for the analysis of forecast indications of the A-scab, Metos (Metos® Pessl Instruments), and RIMpro-Venturia models in connection with meteorological data from the Metos weather station installed in this orchard and to compare them with the actual release dates recorded by the Burkard spore trap. Depending on the year, significant differences were found in the number and intensity of *V. inaequalis* ascospore releases and in their beginning and end dates.

Keywords: apple scab; ascospore discharges; *Malus*; A-scab, Metos and RIMpro-Venturia models

Apple scab caused by the fungus *Venturia inaequalis* (Cooke) G. Winter is one of the most dangerous apple tree diseases in almost all apple production areas worldwide. It occurs annually, mainly on leaves and fruits, causing yield losses. Currently, the most effective way to protect apple trees against this disease is to use fungicides with different mechanisms of action (MacHardy 1996; Rossi et al. 2007; Holb 2009; Hoffmeister et al. 2023). In years with weather conditions favouring the development of apple scabs, up to 20 treatments are required to achieve high protection effectiveness (Meszka

& Masny 2006; Masny 2015). It is also confirmed by data obtained from other countries (Beresford & Manktelow 1994; Holb et al. 2006; Holb 2009; Rancane et al. 2024). Fungicides intended to control apple scab constitute the largest proportion of all pesticides used to protect apple orchards (Penrose et al. 1985; Creemers & van Laer 2006).

The source of primary infections of apple trees are ascospores of the pathogen, the development and release of which have been the subject of research in many centres around the world (Hirst & Stedman 1962, Gadoury & MacHardy 1982a; Gadoury

& MacHardy 1982b; Rossi et al. 2000; Stensvand et al. 2006; Masny & Jankowski 2012; Jankowski & Masny 2019; Masny 2020; Jankowski & Masny 2020). The definition of criteria for infection of apple leaves by *V. inaequalis* allowed for more adequate use of fungicides in the control of apple scab (Mills 1944; Mills & Dewey 1947; Mills & Laplante 1951). This new strategy is based on using fungicides during risk periods, considering, among others, the influence of light and temperature on the daily release of ascospores (Penrose et al. 1985; MacHardy & Gadoury 1989). Period of high relative air humidity until the leaves are wetted (in hours) (Jones et al. 1980), and the use of a coefficient determined based on the duration of leaf wetting and temperature was also important (Schwabe 1980). The distribution of time required for infection of leaves and fruits with individual 'portions' of ascospores settling on these organs in subsequent hours of discharge was also examined in detail, depending on the air temperature and the length of wetting the leaves (Phillion et al. 2019).

A major advance in the simulation of ascospore maturation was the development of simulation models by James and Sutton (1982) and Gadoury and MacHardy (1982a). Next, models of Rossi et al. (2000) and Stensvand et al. (2005) based on the modifications of Gadoury and MacHardy (1982a) were developed. The algorithms of these models are concerned with predicting the dates and proportion of ascospores released in favourable weather conditions (e.g. rainfall) in the primary infection period. These proportions are described by the cumulative temperature function (sum of degree days). Currently used in Europe include models RIMpro-Venturia (Trapman 1994; Trapman 2013), A-scab (Rossi et al. 2007) and Metos (Metos® Pessl Instruments, Austria).

Our research, conducted in 2014–2017, aimed to determine the dates of *V. inaequalis* ascospore releases using the RIMpro-Venturia, Metos and A-scab simulation models. The results were compared with actual spore releases the Burkard spore trap recorded. Using this volumetric method, the number of ascospores on each day of their release was also determined.

MATERIAL AND METHODS

Volumetric assessment of the proportion of *V. inaequalis* ascospore releases. The research

was carried out in the Experimental Orchard of the National Institute of Horticultural Research (IO-PIB) in Dąbrowice near Skierniewice, where various cultivars of apple trees are grown. A Burkard spore trap (Burkard Manufacturing Co Ltd, Great Britain) was installed in a plot of apple cv. 'McIntosh', a common standard for scab high susceptibility (Biggs et al. 2010), not protected against apple scab, was used to record actual ascospore releases. The monitoring was carried out annually, starting from the appearance of numerous colouring (maturing) ascospores in the pseudothecia (usually in the second decade of March) and ending at the turn of June and July, about two weeks after the last spore release was recorded. The release proportion was determined by the number of *V. inaequalis* ascospores caught on Burkard spore trap tapes using a light microscope (200× magnification) (Delta Optical, Poland). The results were compiled for individual hours daily and entered into Excel spreadsheets. The total number of ascospores on a given day and their percentage in relation to all ascospores released during the primary infections were calculated.

Predicted indications of *V. inaequalis* ascospore releases by selected simulation models and analysis of their compliance with actual spore releases. Based on data (average air temperature, leaf wetness, relative air humidity, rainfall) from the Metos weather station (Pessl Instruments GmbH, Weiz, Austria) located in the IO-PIB Experimental Orchard, the release of ascospores of the fungus *V. inaequalis* was predicted using three simulation models.

In the case of the A-scab model, the weather data analysis was performed each year using Excel spreadsheets, starting from February 1 (fixed in each year biofix date set by the model's authors indicating the beginning of the forecast analysis) (Rossi et al. 2007). Predicted ascospore release dates were noted. The number of ascospores was determined and then expressed as a percentage of ascospores on specific dates in relation to the pool of all primary infections predicted during this period in a given year.

Weather data (average air temperature, leaf wetness, relative air humidity and rainfall) were recorded by the Metos weather station and transmitted to the Pessl Instruments Internet server (Metos®) for analysis using the Metos model. The biofix date was established based on microscopic observations of the ascospores release and confirmed by the

Burkard spore trap. However, the method of determining the biofix date based on the linear accumulation of degree days index (<https://metos.at/pl/disease-models-apple/>) proposed by Pessl Instruments was abandoned. This decision was due to the results of our earlier study conducted in 1998–2005. It was found that the values of the degree-day index did not guarantee a precise determination of the date of maturity (readiness) of *V. inaequalis* ascospores for release from pseudothecia (Masny 2005). The dates of all spore releases were determined based on charts generated by the model. Despite the rainfall, the lack of ascospores for at least 14 days from the beginning of June was the basis for the decision to end the prediction.

The start date of prediction with RIMpro-Venturia was based on the biofix date, which was set annually in laboratory conditions after the detection of the first mature ascospores in pseudothecia on the leaves collected from the orchard litter, starting approximately two weeks before apple bud break (BBCH 53) (Trapman 1994; Trapman 2013). The model predicted the intensity of the individual spore release, which was reflected in the number of released ascospores simulated by the model. The conducted analysis provided information on the dates of all ascospore releases and their duration in each year of the study.

As predicted by the models mentioned above, the results of all release dates of *V. inaequalis* ascospores were meticulously analyzed with the actual spore release dates indicated by the Burkard spore trap. This comprehensive analysis ensures the reliability of our findings.

RESULTS

Comparison of volumetric assessment of the dates and proportion of *V. inaequalis* ascospore releases with the prediction of the simulation models. In the years 2014–2017, releases of ascospores started three times in the third decade of March (2014, 2015, and 2017), i.e., in the bud break phase [from end of bud swelling (BBCH 52) and bud burst when green leaf tips enclosing flowers visible (BBCH 53)] and once in the first decade of April 2016, also in the bud break phase (as above). They ended each year in the first decade of June, i.e., in the phase of fruit set growth (BBCH 71) (Table 1).

In individual years of the study, the total number of ascospores trapped on Burkard's spore trap tapes ranged from less than 3 000 (in 2016) to about 12 500 (in 2015) and about 16 200 (in 2014) up to over 59 000 (2017) (Table 2).

It was also found that the number of all spore releases (discharge days) ranged from 26 (in 2015 and 2016) to 28 (2014) up to 32 (2017). However, only 5–7 were the largest spore releases (> 4% of the actual spore releases) when 81–90% of all ascospores releases in a given season were recorded.

Rainfall was most often the direct cause of actual spore release. However, when the rain ended before midnight, ascospores were present in the air of the monitored orchard for several hours the next day. Only 2.2% of all ascospore release dates were not directly related to rain, as they occurred 2–3 days after rainfall in leaf-wetting (dew) conditions and high relative air humidity.

Table 1. The start and end dates of *Venturia inaequalis* ascospore release were predicted by the A-scab, Metos, and RIMpro-Venturia models and recorded with the Burkard spore trap in 2014–2017

Year	Discharge	Burkard	A-scab	Metos	RIMpro-Venturia
		dates of actual and releases*			
2014	first	March 24	March 19	March 24	April 8
	last	June 2	May 16	June 2	June 6
2015	first	March 26	March 26	March 26	April 13
	last	June 9	June 9	June 9	June 27
2016	first	April 6	March 11	March 26	March 26
	last	June 2	May 25	May 26	July 3
2017	first	March 21	March 20	March 21	March 29
	last	June 6	May 25	June 6	July 1

*The date of the first and last ascospore release by the Metos model was scheduled for the day indicated by the Burkard spore trap

Table 2. Number of actual *Venturia inaequalis* airborne ascospores in the orchard during individual phenological phase of apple tree growth in 2014–2017

Phenological phase (BBCH)	2014	2015	2016	2017
Bud swelling and burst (52–53)	93	110	16	198
Mouse-ear stage (54)	5 278	53	1 078	10 465
Tight cluster (55–56)	3 506	1 386	224	28 042
Pink (57)	1 771	4 928	1 261	17 339
Bloom (60–67)	4 991	5 227	259	2 751
Petal fall (69)	42	510	0	0
Fruit set (71)	512	252	31	542
Total	16 193	12 466	2 869	59 337

Predicted indications of *V. inaequalis* ascospore release by selected simulation models and analysis of their compliance with actual spore releases. A detailed comparison of *V. inaequalis* ascospore releases predicted by the A-scab, Metos, and RIMpro-Venturia simulation models and recorded with the Burkard spore trap in individual years showed differences in terms of the individual ascospore releases (Tables 3 and 4).

The RIMpro-Venturia model predicted the start of spore release with a delay of 8 to 18 days (in 2014, 2015, and 2017) or 11 days in advance (2016) and the end – with a delay of 4 to 31 days (in 2014–2017) compared to actual spore releases. The total number of ascospore release dates predicted by this model in 2014–2017 ranged 25–36 (Tables 3 and 4). Among the tested models, RIMpro-Venturia showed the highest number of unpredicted spore releases on dates when actual ascospore release occurred. This situation concerned 5, 7, 8, and 9 dates in 2016, 2014, 2015, and 2017, respectively (Tables 3 and 4). Only 69.2% (in 2015) to 80.8% (in 2016) of ascospore release dates predicted by this simulation model were consistent with the actual spore release dates recorded by the Burkard spore trap (Table 5). On the other hand, this model incorrectly predicted 36 spore release dates (from 4 in 2014 to 15 in 2016) that were not confirmed by volumetric measurements (Table 6).

According to the A-scab model, in 2014, 2016, and 2017, spore releases started 1–36 days earlier and ended 8–17 days earlier. However, in 2015, the starting and ending spore release dates this model predicted were consistent with those recorded by the Burkard spore trap (Tables 3 and 4).

The number of ascospore releases predicted by the A-scab model in 2014–2017 ranged from 25 to 31, approaching the range of actual spore releases recorded with the Burkard spore trap – from 26 to 32. It was also found that this model did not indicate all the dates when actual spore release occurred. This situation concerned three dates in 2015, four in 2016 and seven in 2014 and 2017 (Tables 3 and 4). The consistency between the spore release dates predicted by this model and the actual ascospore releases ranged from 75% to 88.5% (Table 5). Depending on the year, the model incorrectly predicted from one (in 2017) to nine (in 2016) spore release dates not confirmed by volumetric assessments of actual spore releases (Table 6), which was fewer than the models Metos and RIMpro-Venturia.

The Metos model indications were the least discrepant with actual ascospore releases. In the 2016 season, the end date was delayed by 7 days. During the research period, the total number of ascospore release dates predicted by the Metos model ranged from 32 to 44 (Tables 3 and 4). However, this model did not predict several release dates in which *V. inaequalis* ascospores were present in the orchard air. This situation concerned a total of 13 spore releases in the years 2014–2017 (3 in 2014, 4 dates each in 2015 and 2016, and 2 in 2017) (April 18, May 4, and May 10 in 2014; March 26, April 4, May 1, and May 13 in 2015; April 15, April 18, June 1, and June 2 in 2016; April 10, and April 17 in 2017) (Tables 3 and 4). Moreover, compared to the RIMpro-Venturia and A-scab models, this model predicted the most spore release dates not confirmed by volumetric measurements, from 6 in 2014 to 14 in 2017 (Table 6). How-

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Table 3. Actual and predicted *Venturia inaequalis* ascospore releases by A-scab, Metos and RIMpro-Venturia simulation models in 2014 and 2015*

BBCH	Date	Burkard	A-scab	Metos	RIMpro-Venturia	BBCH	Date	Burkard	A-scab	Metos	RIMpro-Venturia
2014						2015					
March						March					
51	19	–	+	–	–	52	26	+	+	+	–
52	23	–	+	–	–	52	27	+	+	+	–
53	24	+	+	+	–	52	28	–	–	+	–
53	25	+	+	+	–	52	29	–	+	+	–
53	26	–	–	+	–	52	30	–	+	+	–
53	27	–	–	+	–	52	31	+	+	+	–
April						April					
54	8	+	+	+	+	52	1	+	+	+	–
54	9	–	–	+	–	53	2	–	+	+	–
54	10	+	+	+	+	53	3	–	+	+	–
55	11	–	+	+	–	53	4	+	–	–	–
55	14	+	+	+	+	53	5	–	+	–	–
56	15	+	+	+	+	53	6	–	+	–	–
56	18	+	+	–	+	54	13	+	+	+	+
56	19	+	–	+	–	55	15	+	–	+	–
56	20	+	+	+	+	56	18	+	+	+	+
56	21	+	–	+	–	56	19	+	+	+	–
57	23	+	+	+	+	57	26	+	+	+	+
60	26	+	+	+	+	57	27	–	–	+	–
60	27	+	+	+	–	57	28	+	+	+	+
May						57	29	+	+	+	+
63	2	+	+	+	+	May					
63	3	+	+	+	+	61	1	+	–	–	–
64	4	+	+	–	–	64	6	+	+	+	+
	7	–	+	+	+	65	7	+	+	+	+
64	8	+	+	+	+	65	8	–	–	+	–
64	9	+	+	+	+	65	10	+	+	+	+
65	10	+	+	–	+	67	13	+	+	–	+
65	12	+	+	+	+	67	14	+	+	+	+
67	13	+	+	+	+	69	17	+	+	+	+
69	15	+	+	+	+	71	19	+	+	+	+
71	16	+	+	+	+	71	20	+	+	+	+
71	17	+	–	+	+	71	21	+	+	+	+
	18	–	–	–	+	71	23	+	+	+	+
71	26	+	–	+	+	71	24	–	–	+	–
	27	–	–	+	+	71	25	+	+	+	+
71	28	+	–	+	+	71	26	+	+	+	+
71	29	+	–	+	–	71	27	–	–	+	–

*(+) recorded or predicted releases of *V. inaequalis* ascospores; (–) lack of ascospore release (Burkard's spore trap) or lack indicated by the simulation model

Table 3. to be continued...

BBCH	Date	Burkard	A-scab	Metos	RIMpro-Venturia	BBCH	Date	Burkard	A-scab	Metos	RIMpro-Venturia
2014						2015					
June						June					
71	2	+	–	+	+	71	9	+	+	+	+
71	6	–	–	–	+	72	12	–	–	–	+
Total		28	25	31	25	72	14	–	–	–	+
						72	19	–	–	–	+
						72	20	–	–	–	+
						73	21	–	–	–	+
						73	22	–	–	–	+
						73	23	–	–	–	+
						74	26	–	–	–	+
						74	27	–	–	–	+
						Total		26	29	32	27

*(+) recorded or predicted releases of *V. inaequalis* ascospores; (–) lack of ascospore release (Burkard's spore trap) or lack indicated by the simulation model

Table 4. Actual and predicted *Venturia inaequalis* ascospore release by A-scab, Metos and RIMpro-Venturia simulation models in 2016 and 2017*

BBCH	Date	Burkard	A-scab	Metos	RIMpro-Venturia	BBCH	Date	Burkard	A-scab	Metos	RIMpro-Venturia
2016						2017					
March						March					
51	1	–	+	–	–	52	20	–	+	–	–
51	15	–	+	–	–	53	21	+	+	+	–
52	21	–	+	–	–	53	22	+	+	+	–
52	22	–	+	–	–	53	23	–	–	+	–
52	26	–	+	–	+	53	29	+	+	+	+
52	29	–	+	–	+	53	30	+	–	+	–
52	30	–	+	–	+	53	31	–	–	+	–
52	31	–	+	–	–	April					
April						54	5	+	+	+	+
52	1	–	+	–	+	54	6	+	+	+	+
53	6	+	+	+	+	54	7	+	+	+	+
53	8	+	+	+	+	55	8	–	–	+	–
54	9	+	+	+	+	55	9	–	–	+	–
54	10	+	+	+	+	55	10	+	+	–	–
54	12	+	+	+	+	55	11	–	–	+	+
54	14	+	+	+	–	55	12	+	+	+	+
55	15	+	+	–	–	56	13	+	+	+	+
56	16	+	+	+	+	56	14	–	–	+	–
56	17	–	–	+	–	56	15	+	+	+	+

*(+) recorded or predicted releases of *V. inaequalis* ascospores; (–) lack of ascospore release (Burkard's spore trap) or lack indicated by the simulation model

Table 4. to be continued...

BBCH	Date	Burkard	A-scab	Metos	RIMpro-Venturia	BBCH	Date	Burkard	A-scab	Metos	RIMpro-Venturia
2016						2017					
April						April					
56	18	+	+	–	+	56	16	+	–	+	–
56	19	–	–	+	–	56	17	+	–	–	–
56	20	–	–	+	–	56	18	+	+	+	–
57	24	+	+	+	–	57	21	–	–	+	–
57	27	+	+	+	+	57	22	+	+	+	+
57	28	+	+	+	+	57	23	+	+	+	+
60	29	+	–	+	–	57	24	–	–	+	–
60	30	–	–	+	–	57	25	+	+	+	+
May						57	26	+	+	+	+
61	2	–	–	+	–	57	27	+	+	+	+
62	3	+	+	+	+	59	28	+	+	+	+
64	4	+	+	+	+	59	29	+	+	+	+
64	5	–	–	+	–	May					
64	6	–	–	+	–	62	2	+	+	+	+
64	7	–	–	+	–	62	3	–	–	+	–
65	8	+	+	+	+	63	4	+	+	+	+
65	9	+	+	+	+	64	5	+	+	+	+
65	10	–	–	+	–	64	6	–	–	+	–
65	11	+	+	+	+	65	7	+	+	+	+
65	12	+	+	+	+	65	8	+	+	+	+
65	13	+	+	+	+	65	9	–	–	+	–
65	14	+	+	+	+	67	13	–	–	+	–
65	15	+	+	+	–	67	15	–	–	+	–
67	17	–	–	+	–	71	24	+	+	+	+
71	25	+	+	+	+	71	25	+	+	+	–
71	26	+	–	+	+	71	26	+	–	+	+
June						71	29	+	–	+	–
71	1	+	–	–	+	June					
71	2	+	–	–	+	71	4	+	–	+	+
72	8	–	–	–	+	71	5	–	–	+	–
72	9	–	–	–	+	71	6	+	–	+	+
72	10	–	–	–	+	72	12	–	–	–	+
73	15	–	–	–	+	73	14	–	–	–	+
73	16	–	–	–	+	73	16	–	–	–	+
73	17	–	–	–	+	73	17	–	–	–	+
73	20	–	–	–	+	73	28	–	–	–	+
73	25	–	–	–	+	74	30	–	–	–	+
73	26	–	–	–	+	July					
73	30	–	–	–	+	74	1	–	–	–	+
July						Total					
74	3	–	–	–	+	32	26	44	31		
Total		26	31	32	36						

Table 5. Consistency of predicting *Venturia inaequalis* ascospore release dates using simulation models (%)

Year	Simulation model						
	Burkard	A-scab		Metos		RIMpro-Venturia	
	A	B	C	B	C	B	C
2014	28.0	21.0	75.0	25.0	89.3	21.0	75.0
2015	26.0	23.0	88.5	23.0	88.5	18.0	69.2
2016	26.0	22.0	84.6	22.0	84.6	21.0	80.8
2017	32.0	25.0	78.1	30.0	93.8	23.0	71.9

A – number of actual spore release dates; B – number of ascospore releases predicted by the mentioned models on the actual release dates; C – compliance of indications (in %)

Table 6. Number of incorrectly predicted release dates for *Venturia inaequalis* ascospores not in accordance with Burkard's spore trap indications

Year	Simulation model		
	A-scab	Metos	RIMpro-Venturia
2014	4 (16.0)*	6 (19.4)*	4 (16.0)*
2015	6 (20.7)	9 (28.1)	9 (33.3)
2016	9 (29.0)	10 (31.3)	15 (41.7)
2017	1 (3.8)	14 (31.8)	8 (25.28)

* The percentage of predicted incorrect spore releases compared to Burkard's indications is given in parentheses

ever, this simulation model proved to be the most accurate because 84.6% (in 2015 and 2016) to 93.8% (in 2017) of the predicted ascospore releases were consistent with the actual spore releases indicated by the Burkard spore trap (Table 5).

DISCUSSION

Depending on the year of our study, differences were found in the number and intensity of *V. inaequalis* ascospore releases and the dates of their starting and ending. According to Burkard spore trap data, the release of ascospores began in three years (2014, 2015, and 2017) in the third decade of March and in one year (2016) in the first decade of April. The end of the spore releases was annually from June 2 to June 9. All release days ranged from 26 (2015 and 2016) to 32 (2017). The occurrence of rainfall was the main factor for the release of ascospores. It was found that only 2.2% of all spore release dates were not directly related to rain, as they occurred 2–3 days after rainfall in conditions of leaf wetting (dew) and high relative air humidity.

The release of *V. inaequalis* ascospores in dew conditions was also found in south-eastern Norway, during which from 1.4% to 26.9% of these spores were recorded in the orchard air, out of all

those recorded in a given season (Stensvand et al. 1998). In Italy, however, Rossi et al. (2001) found that dew was always insufficient for spore release to occur without rain. It was also emphasized that wind is important in spreading the primary source of infection over longer distances but is a factor of little importance within the orchard (Rossi et al. 2006). The results of the research of Alt and Kolar (2010) in Germany indicate that atmospheric humidity or moisture from dew does not have an inductive effect on ascospore release. The authors found that raindrops cause vibrations of wetted leaves, which induce release. Noteworthy is the research by Wallhead et al. (2017), who emphasized that abundant rainfall favouring spore release at the beginning of the period of primary apple leaf infections reduced the number of mature ascospores in pseudothecia and, at the same time, ended their release earlier. Other authors also draw attention to the importance of rain in initiating the release of ascospores, believing that most of them are released within the next hours after its end (Aylor & Sutton 1992). Rossi et al. (2001) determined that the threshold value of rainfall lasting from one to several hours, continuously or interrupted for a maximum of two hours, was 0.2 mm/h. Hindorf et al. (2000) found that alternating wet and dry periods favour the release and spread of as-

cospores, and the presence of light is an important factor stimulating spore release. In their opinion, the release of ascospores is characterized by a clear circadian rhythm, with the majority of these spores in the conditions of North Rhine and Westphalia (Germany) being realized between 10 am and 6 p.m. Stensvand et al. (2009) also emphasized that light is very important for intensity of ascospore releases during wet conditions. They observed cumulative ascospore release in 3 hours after sunrise at a level of 0.8%, 3.0 % and 8.1% (at temperatures 0–5 °C, 5–10 °C and above 10 °C respectively) and their increase up to 50% at 8–11 h after sunrise. Our research also observed all the relationships mentioned above, regardless of the year. Moreover, Ehlert et al. (2017) proved that infrared radiation induces *V. inaequalis* to release spores. The research results of these authors indicate that artificial illumination by such light during the night was correlated with an increase of up to 46% of ascospores released overnight in the field.

In our studies conducted in Central Poland, the first mature pseudothecia were observed in early spring (at the beginning of March 2016 at the earliest). We also found no relationship between the rainfall intensity in the autumn and the abundance of pseudothecia in the next spring. On the other hand, the thickness of the snow cover in winter influenced the increase in the number of pseudothecia on the leaf surface (Masny 2020). In this context, it is worth adding that in Great Britain, heavy rainfall in November and high temperatures in spring resulted in the fastest ripening of pseudothecia and, consequently, developing ascospores. However, mature pseudothecia were most abundant, with low temperatures in November and low rainfall in spring (Jeger & Butt 1983).

Analyzing the total rainfall occurrence during the spore release periods in individual years and the average air temperature during these periods in Central Poland, it should be mentioned that the highest rainfall was in 2017 (197 mm), and the lowest was in 2015 (104 mm). However, the average temperature values were similar, i.e. the lowest was in 2014 (11.6 °C), and the highest in 2017 (12.5 °C). It was shown that ascospores' maturation and release were determined by favourable conditions for short periods, e.g. up to several days (Masny 2020).

Earlier research conducted in central California (USA) showed that the first ascospore release occurred just before the "silver-tip" phase (the end

of the swelling of apple buds, when their lightly coloured scales are densely covered with hairs) in the earliest developing apple cultivars (Moller 1980). Maximum spore release from the leaves of six apple cultivars ('Golden Delicious', 'Granny Smith', 'Ruby', 'Summerred', 'Spur Rome' and 'Winesap') took place from the green tip of the first leaf on the 'Spur Rome' cv. to the opening of the first flowers on 'Summerred' and occurred during the same week. However, the ascospore release period lasted over a month and ended before most cultivars reached full flowering. Individual cultivars differed significantly in terms of the total number of ascospores produced. In the case of the 'Ruby' cultivar, they were found to be only 4.7% compared to all those of the leaves of 'Winesap' (Moller 1980).

Comparing the results of our four-year study (also considering other years whose results were not published) with the results obtained from studies in south France (Avignon area), it can be concluded that the differences in the dates of the first and last ascospore release as well as the total number of ascospore releases were high (Roubal & Nicot 2016). Thus, during 13 years (1996–2008) of observations in France, the first ascospores were recorded between February 23 and April 8, while in Poland, they were recorded from March 21 to April 6. The dates of the last release of ascospores also differed in individual years and fell between May 5 and June 10 (France) and June 2–9 (Poland). Observations in 2000 in France are noteworthy, in which after a 40-day drought that occurred after the first observation of mature ascospores, as much as 41% of the total ascospore potential was released during the first rain. In individual seasons of our research, the total number of ascospores recorded on Burkard's spore trap tapes ranged from 3 000 to over 59 000. However, in the dry climate of France, less abundant spore releases were recorded, and the total number of ascospores counted during the season ranged from less than 500 to over 48 000 (Roubal & Nicot 2016).

In six-year studies (1994–1999) conducted at various locations in Victoria (Australia), *V. inaequalis* ascospores were recorded within three months from the green tip phase of the first apple leaf, i.e., from the beginning of September to the end of November (Villalta et al. 2002). In the majority of seasons, most ascospores were recorded during flowering, but in years with prolonged dry periods (> 7 days) during flowering,

ascospore release was significantly delayed. Ascospore release was mainly caused by moisture from rainfall and recorded during dew periods. In all years, most spore releases occurred under daylight, and less than 17.5% of ascospores were recorded during darkness (e.g., from 7 pm to 5 am) (Villalta et al. 2002). In Central Poland, most spore releases were recorded from a half-inch green phase in 2014, through a tight cluster in 2017 and pink in 2016, to bloom in 2015.

Our analysis of the start and end dates of *V. inaequalis* ascospore releases predicted by all tested models compared with those recorded by the Burkard spore trap indicates some differences. Data shown by the A-scab model were consistent with the actual spore release start and end dates only in 2015. In 2014, 2016, and 2017, spore release started (1–26 days) and ended (8–17 days) earlier than predicted. However, research conducted in Brazil (Vacaria region) in 2009–2012 showed that the A-scab model, compared to the models of Gadoury and MacHardy (1982a, 1982b), Beresford (1999) and Alves and Beresford (2013), is the most reliable and reflected the actual spore release recorded using microscope slides of the method of Manktelow and Beresford (1995). It should be emphasized that the availability of A-scab model algorithms allows its calibration. We believe this creates great prospects for its practical use, especially after the model is developed in digital form.

In the case of the RIMpro-Venturia model, the start dates of ascospore release were predicted in Poland with a delay of 8 to 18 days (in 2014, 2015, and 2017) or 11 days in advance (2016), and the completion dates – with a delay of 4 to 38 days (in 2014–2017) compared to actual discharge. The late predicted indication of the first spore release also has consequences concerning the actual maturity of *V. inaequalis* ascospores and their readiness for release. Such information is precious for the timely initiation of protection, especially in the presence of a high infection source and the need to perform the first curative treatment. Moreover, in practice, the first curative treatment is allowed only when the level of the source of infection is low. However, if information about the end of spore releases is delayed, continuing protection against primary apple tree infections will be unnecessary. Meanwhile, in Germany, in studies on Lake Constance (1994–1996), entirely accurate model RIMpro-Venturia indications were found regarding ascospore release in 1995 and

1996 compared to those recorded using microscope slides suspended 4 mm above heavily infected apple leaves (Triloff 1997). RIMpro-Venturia slightly overestimated the spore release amount at the season's beginning and end. However, all predicted spore releases were consistent with the actual ones regarding dates and proportions (about all ascospores released in the season).

In 2002 and 2003, detailed studies were carried out at two locations (Güttingen and Wädenswil) in Switzerland, comparing the ascospore release simulated by the RIMpro-Venturia model and the actual spore release determined by Myco-Trap (Sacchelli & Siegfried 2004). It was found that only in the first year was there an agreement between the start and end date of ascospore release of *V. inaequalis* indicated by Myco-Trap compared to that predicted by RIMpro-Venturia. In the second year, however, only the correct end date of spore release was confirmed in Güttingen, whereas in Wädenswil, it was predicted about one week earlier.

A three-year study conducted in 4 orchards in the south of New England (USA) showed high compliance (occasionally a 3-day delay) of the first ascospore release predicted by the RIMpro-Venturia model compared to the assessment of ascospore maturity carried out using the Petri plate assay method (PPA) (Garofalo 2019). However, there was variation in the last spore release predicted by the model, i.e., three days later to over five weeks later than indicated by the results of tests. In turn, research by Pedersen et al. (2006) carried out in Denmark in 2002–2004 showed that at the beginning of the season, the dates and volumes of ascospore release predicted by the RIMpro-Venturia model did not reflect the actual spore release. The low temperatures in early spring probably contributed to the discrepancies found.

Comparison of the start and end dates of *V. inaequalis* ascospore release predicted by all three models with the actual spore release dates recorded using the Burkard spore trap in Central Poland indicates that the minor discrepant indications were provided by the Metos model, which is undoubtedly related to the data obtained using the Burkard spore trap (biofix). During the four-year study (2014–2017), in 2015 and 2017, this model showed a 1-day delay in the start of spore release and a 7-day advance at the end of spore release, respectively, compared to the actual recorded data and

in the remaining years the forecast data was consistent with real ones.

Among the other simulation models studied, the spore release start and end dates simulated by the A-scab and RIM-pro Venturia models were largely inconsistent with the real ones. However, it should be emphasized here that all the models are degree-day. In spring periods with low temperatures, this may not be sufficient for correctly determining the start of spore release. During critical high temperatures at the end of ascospore release, it may also cause errors. Roubal and Nicot (2016) showed that under extreme weather conditions, a continuous nonlinear thermal time scale can be more effective than a degree-day scale, even though their model is a model predicting pseudothecia maturity and not ascospore release dates. In the analysis of the accuracy of disease risk prediction, the potential of the source of infection is also worth considering, especially at the beginning and before the end of ascospore release. Many factors, including the above mentioned environmental factors, influence this. Detailed knowledge of the relationships between individual factors allows for improving prediction systems. It is also worth considering the different climatic conditions where the system is used, as it was originally developed in completely different conditions. In this context, a major challenge is also the ongoing climate change.

In Lithuania, in 1999–2002, the Metos model was useful for optimizing the timing of treatments to protect apple trees against scabs. According to the model's recommendations, studies have shown high or equal effectiveness in protecting apple trees against apple scabs in schematic and integrated programs. It is worth noting that the biofix of this model was determined on the day when the sum of degree days (base = 0 °C) was 500, not to mention the phenology of the apple trees (Raudonis et al. 2003). In later studies (2007–2008), Raudonis and Valiuškaite (2009) confirmed that the Metos model predicted ascospore releases and periods of infection caused by ascospores and conidia, depending on air temperature, leaf wetting time and relative humidity.

To sum up, it should be emphasized that there is little detailed data in the available literature comparing predicted indications for the release of *V. inaequalis* ascospores at the same site in different models for central and eastern Europe regions, including Poland.

CONCLUSIONS

(i) Comparison of the start and end dates of *V. inaequalis* ascospores release, predicted by the A-scab, Metos, and RIMpro-Venturia models, with actual spore releases indicates that, in most cases, there is no complete agreement between them:

- the Metos model,
- compliance with the spore release start dates predicted by the A-scab model with actual spore releases was shown in only one year,
- the most discrepant predicted indications were obtained from the RIMpro-Venturia model.

(ii) Different weather conditions in different locations, even in a small orchard region, may cause differences in the readiness dates of *V. inaequalis* ascospores for the first release. To eliminate this variability, it is necessary to analyze the forecast for each location where meteorological data are recorded.

(iii) The A-scab simulation model seems the most useful in orchards, provided that calibration is performed annually (establishing an individual biofix date for each season and location).

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