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FACULTY OF PLANT PROTECTION AND AGROECOLOGY

DEPARTMENT OF PHYTOPATHOLOGY

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EPIDEMIOLOGY AND CONTROL OF CHERRY LEAF SPOT IN SWEET AND SOUR CHERRY (*BLUMERIELLA JAAPII*)

DISSERTATION ABSTRACT

For awarding the educational and scientific degree "Doctor"
Professional field: 6.2. Plant protection
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Scientific supervisor:
Ch. Assist. Prof. Dr. Zvezdomir Zhelev

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The dissertation is written on 184 pages, contains 41 tables and 59 figures. The cited literature includes 310 sources, of which 43 are in Cyrillic and 267 are in Latin.

The studies were conducted during the period 2018–2020 at the Department of Phytopathology and the Center for Integrated Plant Disease Management at the Agricultural University-Plovdiv in sweet and sour cherry plantations in the districts of Plovdiv, Stara Zagora, and Sofia.

The dissertation was discussed and proposed for defense at a meeting of the Department Council at the Department of Phytopathology at the Agricultural University-Plovdiv, with protocol No. 14 of 29.10.2024.

The defense of the dissertation will take place on ………………………………… from ……………… in ………………………………… of the Agricultural University-Plovdiv at an open meeting of a scientific jury, approved by order of the Rector of the Agricultural University with order No. RD-16-1330 of 22.11.2024, composed of:

Chairman:

Prof. DSc. Mariyana Borisova Nakova

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The materials for the defense are available on the website of the Agricultural University - Plovdiv https://www.au-plovdiv.bg/en/ and in the library of the Agricultural University - Plovdiv, Mendeleev Blvd. No. 12.

I. INTRODUCTION

One of the most common fungal diseases of cherries around the world is Cherry leaf spot (CLS), which is caused by *Blumeriella jaapii* (Rehm) Arx. Due to the lack of resistant varieties with organoleptic and technological characteristics comparable to those currently grown, control is mainly through fungicides and sanitation. As a result of the long-term use of systemic fungicides, new resistant races of the pathogen have emerged, which poses a major threat to the fruit production in some parts of the world. The issue of reducing the amount of pesticides used is addressed in the development of the European Commission's (EC) Farm to Fork Strategy, which is part of the European Green Deal. Forecasting models implemented in the Decision Support System (DSS) are considered one of the main methods for reducing chemical spraying.

II. OBJECTIVE AND TASKS

The objective of this dissertation is to study new epidemiological features and possibilities for forecasting and control of the Cherry leaf spot disease caused by *Blumeriella jaapii* (Rehm) Arx.

To achieve this goal, we set ourselves the following specific tasks:

- To study the symptoms of the disease in Bulgaria.
- To study the amount of ascospores (AS) and spring macroconidia (SPM) present in the air and ready to infect.
- To describe the rate of decomposition in overwintered leaves as a source of inoculum.
- To validate the disease prediction model created by the University of Michigan (USA) using potted trap plants.
- To test a chemical control strategy by applying fungicides at different infection index.

III. MATERIALS AND METHODS

1. Identification

1.1. Symptoms of CLS in sweet and sour cherries

Symptoms of the disease are described after monitoring its development in production plantations under natural conditions in three regions of the country. Symptoms of CLS are compared with other similar ones caused by pathogens or pests. The disease is described by percentage incidence,.

1.2. Isolation and cultural characteristics

For pathogen isolation, the methodology of Guo et al. (2018) was used. Heavily infected leaves with CLS from the sweet cherry cv. Van were collected in the first half of September (2018-2020) from the orchard at the Center for Integrated Plant Disease Management at the Agricultural University-Plovdiv and stored in a refrigerator at 4°C until use. The cultural characteristics of 10 isolates were determined by cultivation on MMA. The morphological characteristics of the pathogen were determined by microscopy of its structures (ascospores, spring and summer macroconidia, microconidia) from naturally infected plant parts, as well as mycelium and summer macroconidia (SMM) from pure culture. The data were analysed by the method of Zaitsev (1984) using the MS Excel 365 program (Microsoft Corporation, Redmond, Washington, USA).

2. Epidemiology

2.1. Seasonal and diurnal pattern of ascospores and spring macroconidia release

A 7-day spore trap (Burkard Manufacturing Co. Ltd.) was used to study the seasonal and diurnal patterns of AS and SPM release in Plovdiv, Brestnik, Krichim, Gabarevo, and Botevgrad from 2018 to 2020. An automatic weather station at each location provided data on temperature (T, °C), precipitation (P, mm), relative humidity (RH – relative humidity, %) and leaf wetness duration (LW, h).

Data analysis was performed using MS Excel 365 (Microsoft Corporation, Redmond, USA). Hourly and daily data from spore traps and weather stations was used to analyze the seasonal and diurnal patterns of spore release at each location.

2.2. Development of apothecia and spring acervuli under laboratory conditions

The experiment was carried out according to the methodology used for other ascomycete fungi (Eikemo et al., 2011; Gadoury and MacHardy, 1982a). In 2019 and 2020, samples were collected from unsprayed trees from an orchard planted with the Van/GiSelA 6 rootstock combination located in the CIUBR at the AU-Plovdiv. The methodology consisted of preparing the samples for testing and incubating them in the dark in a thermostat at a temperature of 22℃ (± 0.5℃). Testing leaf discs and fruit peduncles by washing with tempered distilled water every 2-3 days (≈70 degree days (DD)); filtering the liquid through a vacuum filtration system; preparing a temporary microscope slide; examining the samples using a light microscope under x400 magnification (Primo Star, Zeiss, Germany). The number and type of spores were recorded based on their morphological characteristics (Higgins, 1914). When the first mature spores were counted, the accumulation of degree days (DD) was initiated relative to a temperature base (TB) of 0℃. The experiment was terminated when no mature spores were released from fruiting bodies for approximately 100 DD. Data analysis was performed using MS Excel 365 (Microsoft Corporation, Redmond, Washington, USA).

2.3. Dynamics of leaf litter decomposition after leaf fall

The experiment was conducted in 2018-2020 in Plovdiv, Brestnik, Krichim, Gabarevo, and Botevgrad. The dynamics of leaf litter decomposition under natural conditions were assessed according to the methodology of Gadoury and MacHardy (1986). Surveys were conducted from late November (after leaf fall) to mid-July every 14-16 days. Data analysis was performed using MS Excel 365 (Microsoft Corporation, Redmond, Washington, USA).

2.4. Development of CLS during the season

The development of the disease was monitored in the period 2018-2020 in five locations in the country, where spore traps were deployed in the respective year. For this purpose, route surveys were conducted at a certain interval. On each date, the phenological stage of the host was recorded using the standard BBCH scale of Meier et al. (1994).

To analyse the dynamics of the development of CLS during the season, the MS Excel 365 programme (Microsoft Corporation, Redmond, Washington, USA) was used. For each date, average values of incidence and premature defoliation are presented. To study the relationship between meteorological factors and the development of CLS, the R programme, version 3.6.3 (2020-02-29) (Team, 2013), with the main plm package, was used. A two-way fixed effects model of panel data was selected.

2.5. Validation of a predictive model using potted trap plants

The research was conducted in the period 2018-2019 at the Centre for Integrated Plant Disease Management at the Agricultural University of Plovdiv. The experiment was carried out according to the methodology of Philion et al. (2009), which was modified by us. 60 two-year-old trees of the susceptible varieties Van and Heymanova Konserva (Fanal) were planted in containers with a volume of 30-50 l.

The experiment began when the first bract leaves (BBCH 11) appeared at 6 to 8 days old, which is the earliest susceptible stage (Gleason et al., 2021). In 2018, three plants of each variety were placed in the row spacing of the cherry plantation before predicted rainfall. The trees were returned to the tunnel and replaced with others if a dry period of at least 8 hours followed the end of the rainfall, in which the leaves dried under natural conditions in the plantation. From the 5th to the 21st day after their collection in the polyethylene tunnel, they were recorded at two-day intervals for the appearance of symptoms.

In 2019, potted trap plants were positioned around a plot of naturally overwintered infected leaves that had been prepared in the fall of the previous year. Trees were placed around this plot under the same dry season duration definitions described for 2018. Over the two years, three plants of each variety were placed under the polyethylene tunnel to serve as controls.

An automatic weather station model, iMETOS IMT200 (Pessl Instruments, Weiz, Austria), located approximately 50 m from the orchard provided meteorological data. The experiment ended at the end of June, when fruiting bodies from naturally infected leaves that had overwintered were examined under a microscope and found to have empty apothecia and spring acervuli.

3. Control

3.1. Characteristics of the experimental plot and experimental methodology

The field trials were conducted during the period 2019–2020 in a cherry orchard with cv. Van located in the Centre for Integrated Plant Disease Management at the Agricultural University of Plovdiv. The experiments were set up according to a randomised block design (EPPO PP 1/152, 2012). During both years, fungicides were applied according to the forecasting model of Eisensmith and Jones (1981a, b) programmed in the DSS RIMpro BV (Netherlands). In 2019, variants that were treated before the predicted infection period (EFI - Environmental favorability index) were: (1) low index - EFI≥14; (2) medium index - EFI≥28; and (3) high index - EFI≥56 and untreated control. In 2020, variants included treatment before the predicted infectious period with (1) low index - EFI≥14; and (2) medium and high index - EFI≥28-56 and untreated control.

Depending on the stage of the crop and weather conditions during the season, registered fungicides with different mode of action were used. Evaluation of the effect of treatments against CLS was determined by examining the variants and untreated control during the season. The total number of leaves/fruit peduncles and total number of infected leaves/fruit peduncles were recorded. The severity was assessed visually on a scale of 0-100%.The R programme version 4.1.2 (2021-11-01) was used to analyse the experimental data. The following main packages were applied: agricolae (1.3.5); multcomp (1.4.18); MASS (7.3.54); stats (4.1.2); ggplot2 (3.3.5); dplyr (1.0.7).

IV. RESULTS AND DISCUSSION

1. Identification

1.1. Symptoms of CLS in sweet and sour cherries

The monitoring of cherry and sour cherry plantations in the period 2018-2020 allows for a detailed description of the symptomatic picture of CLS. Usually, the first symptoms appear between the end of April and the second ten days of May. In cherry, the spots on the upper side of the leaf are small, with a diameter of 1-3 mm and red to purple in color (Fig. 1a, b). Occasionally, they form narrow stripes along the periphery or the main vein of the leaf (Fig. 1). In wet weather are formed acervuli with summer macroconidia (SMC), forming a specific wick (Fig. 1d). In sensitive varieties, about 14 days after the appearance of necrotic spots, the leaves begin to fall prematurely.

Symptoms of CLS may be observed as early as the appearance of bract leaves (Fig. 2). According to Gleason et al. (2021), early season infections are a prerequisite for the occurrence of epiphytotic.

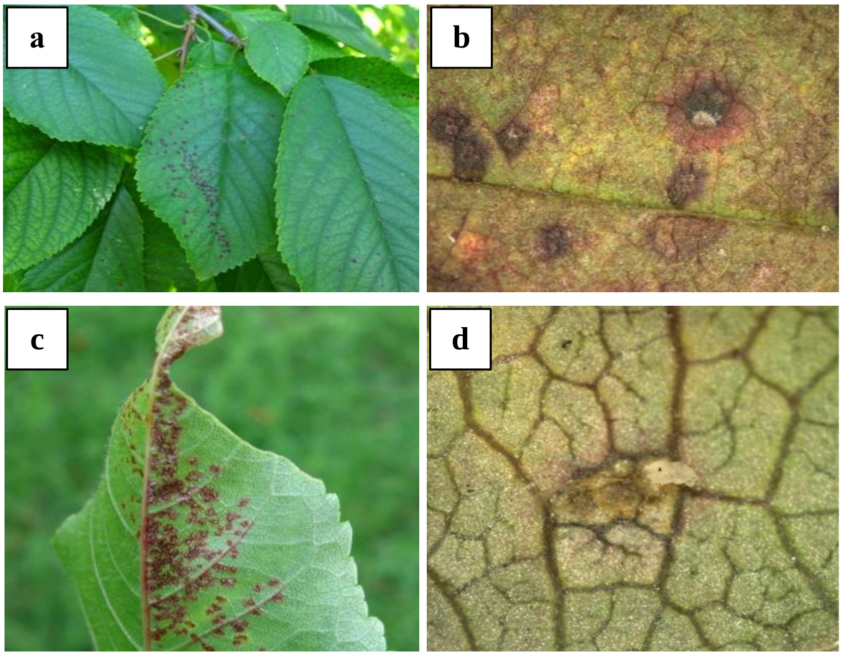

Figure 1. Symptoms of CLS on the adaxial (a, b) and the abaxial side (c, d) of a sweet cherry leave (cv. Kordia).

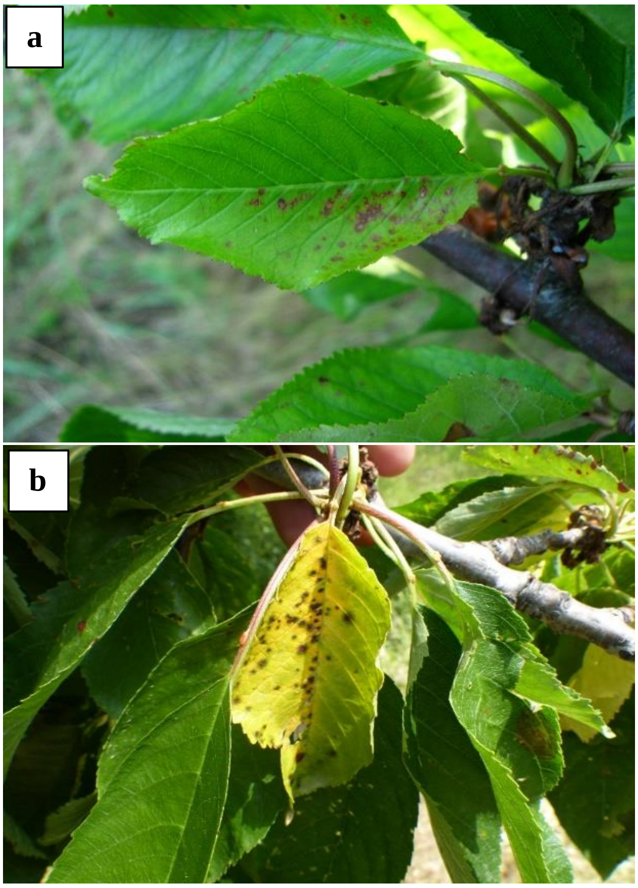

Figure 2. Symptoms of CLS on sweet cherry bract leaves (cv. Van) on 20.5.2020 (a) and 19.6.2020 (b), Location: Krichim.

In severe cases, the tissues around the spots turn yellow and become chlorotic, and the leaves fall prematurely in the second half of July. As a result, the trees overwinter more difficultly and suffer from low temperatures (Wilcox, 1993).

In addition, symptoms have also been observed on the fruit peduncles of cherries and sour cherries. The lesions are in the form of elongated spots measuring 1-2 mm and initially red to violet in colour. Subsequently, the tissues necrotize, and only a purple-violet ring remains around the lesions.

1.2. Isolation and cultural characteristics

On MMA medium, 10 isolates of the pathogen were obtained, designated Ch 4, Ch 8, Ch 20, Ch 24, Ch 29, Ch 33, Ch 47, Ch 59, Ch 63, Ch 70. The first visible colonies were found 14 days after inoculation. The color on their upper side varies from white to "dirty" white. On the lower side, the isolate is initially light brown, later changing to dark brown. The mycelium of the fungus is dense and slimy, with an alternating density and radially embossed structure (Fig. 3).

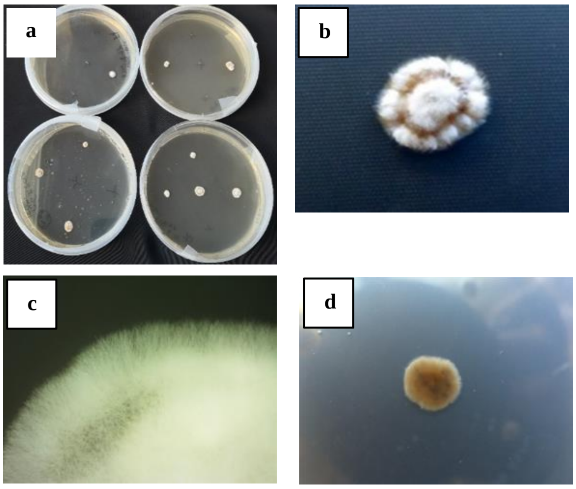

Figure 3. Morphology of a 21-day culture of *Cylindrosporium hiemalis* (isolate Ch 8); Culture medium: MMA; Origin: Plovdiv; cv. Van; Colonies (a); upper side (b); periphery (c); lower side (d).

The colonies of the fungus on artificial culture medium are small in size, with a medium to coarsely serrated periphery. Growth from 14 to 21 days is extremely slow, although it accelerates slightly from 21 to 35 days (Table 1).

Table 1. Diameter of *C. hiemalis* (isolate Ch 8) on MMA

Days after inoculation	**Limit values (min–max), mm**	**Mean (mm) and standard error**	**Standard deviation**
14	2.30 - 6.70	5.00±0.43	1.38
21	2.70 - 7.30	5.60±0.46	1.46
28	6.60 - 13.20	9.90±0.58	1.84
35	10.60 - 15.40	12.50±0.48	1.51

1.3. Morphological characterization of the pathogen

The hyphae are hyaline and branched, and the segments are short, smooth, and thick-walled. Their average thickness is 2.5 μm, close to that reported by other authors (Table 2). The cultural characteristics of the fungus in the present study coincide with the data from other studies on the subject (Velichkova, 1983; Khan et al., 2014; Khan et al., 2016).

Table 2. Biometric characteristics of hyphal thickness of *C. hiemalis* (isolate Ch 8)

Substrate/source of information	**Limit values (μm , min – max)**	**Mean value (μm) and standard error**	**Standard deviation**
MMA (own data)[a]	2.00-3.50	2.50 ±0.07	0.71
Velichkova (1983) [b]	2.02-2.44	2.10*	–
Malt agar (Velichkova, 1983)	–	2.06*	–
Potato agar (Velichkova, 1983)	–	2.18*	–
PDA[c] (Khan et al., 2016)	3.45-7.36	4.87*	–

* Standard error data are missing. [a] MMA - Modified malt agar; [b] The values were determined when cultivating on nutrient media of different compositions. [c] PDA – Potato dextrose agar.

All observed samples contained filamentous, curved, thinned towards the tip, hyaline-colored summer macroconidia (SMM) with two or three septa (Fig. 4). It is noticeable that the length of the SMC is greater in spores taken directly from the MMA (Table 3). This is probably due to a constant temperature and easy accessibility to the fungus components in the nutrient medium. Variation in the length of the spores is greater compared to their width.

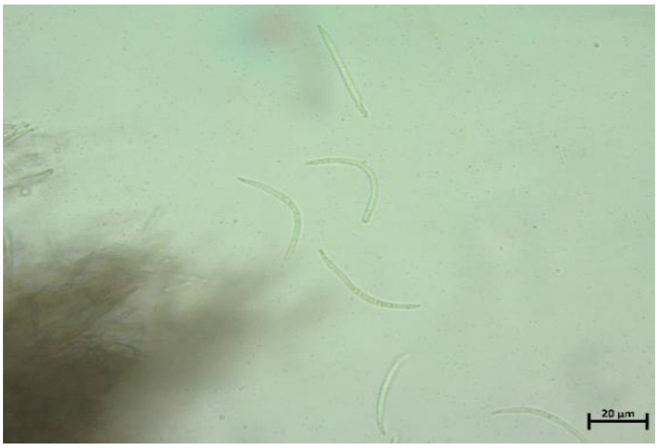

Figure 4. Summer macroconidia of *C. hiemalis* (isolate Ch 8) formed on MMA.

Table 3. Biometric characteristics of summer macroconidia of *C. hiemalis* (isolate Ch 8)

Substrate/source of information	Size (µm)	Limit values (µm , min – max)	Mean value (µm) and standard error	Standard deviation
MMA (own data) [a]	length	50.00-75.00	62.80 ±0.64	6.40
	width	2.50-5.00	3.20 ±0.08	0.84
Naturally infected leaves (own data)	length	40.00-72.50	52.80±0.89	8.91
	width	2.50-5.00	3.10±0.06	0.59
MA (Velichkova, 1983) [b]	length	12.50-84.37	21.84–48.90*	–
	width	5.00-10.62	6.16–6.81*	–
OA (Khan et al., 2014) [in]	length	55.50–72.70	–	–
	width	2.00–3.50	–	–
PDA (Khan et al., 2016)	length	35.50-43.60	39.01*	–
	width	2.30-3.50	2.89*	–

* Standard error data missing.; [a] MMA – Modified malt agar.; [b] MA – Malt agar.; [c] OA – Oat agar with added cherry leaf decoction; [d] PDA – Potato-dextrose agar.

In the isolates from MMA, the SMC was found to be longer on average (62.8 µm / ± 0.64) than what Velichkova found (21.84–48.90 µm). According to Magie (1935) cited by Velichkova (1983), it is possible to vary the morphological features of the pathogen in pure culture. Velichkova (1983), citing Magie (1935), asserts that the pathogen's morphological features can vary in pure culture. In the present experiment, no significant morphological differences between the individual isolates were found.

Morphological features of SMC (Fig. 5; Table 4), taken from plant material, show similarity to descriptions in other studies (Velichkova, 1983; Higgins, 1914).

Figure 5. Summer macroconidia of *C. hiemalis* in naturally infected leaves.

Table 4. Biometric characteristics of summer macroconidia of *C. hiemalis* found in naturally infected leaves

Source of information	**Size (µm)**	**Limit values (µm , min-max)**	**Mean value (µm) and standard error**	**Standard deviation**
Own data	length	40.00 - 72.50	52.8±0.89	8.91
	width	2.50 - 5.00	3.1±0.06	0.59
Velichkova (1983)	length	29.30-77.80	55.70	–
	width	4.00-9.00	6.49	–
Higgins (1914)	length	45.00-65.00	–	–
	width	2.50-4.00	–	–

* The data comes from a table that appears in the dissertation's full text.

Microscopy of summer acervuli at the end of August revealed the presence of microconidia, which, in terms of their morphological features, have similar characteristics to those reported by other authors (Velichkova, 1983; Higgins, 1914; Khan et al., 2014; Khan et al., 2016). They are elongated-elliptic, hyaline, and unicellular; their sizes are similar to those previously published in our country and abroad (Table 5).

Table 5. Biometric characteristics of microconidia of *C. hiemalis* found in naturally infected leaves

Source of information	**Size (µm)**	**Limit values (µm , min-max)**	**Mean value (µm) and standard error**	**Standard deviation**
Own data	length	3.00-5.00	4.30±0.06	0.62
	width	1.25-2.50	1.50±0.04	0.42
Velichkova (1983)	length	4.50-5.25	5.10	–
	width	1.75-3.00	2.41	–
Higgins (1914)	length	4.00-5.00	–	–
	width	–	1.50*	–

* Standard error data is missing. ** The data comes from a table that appears in the dissertation's full text.

The ascospores are elongated-fusiform, hyaline with 1-2 septa (Fig. 6).

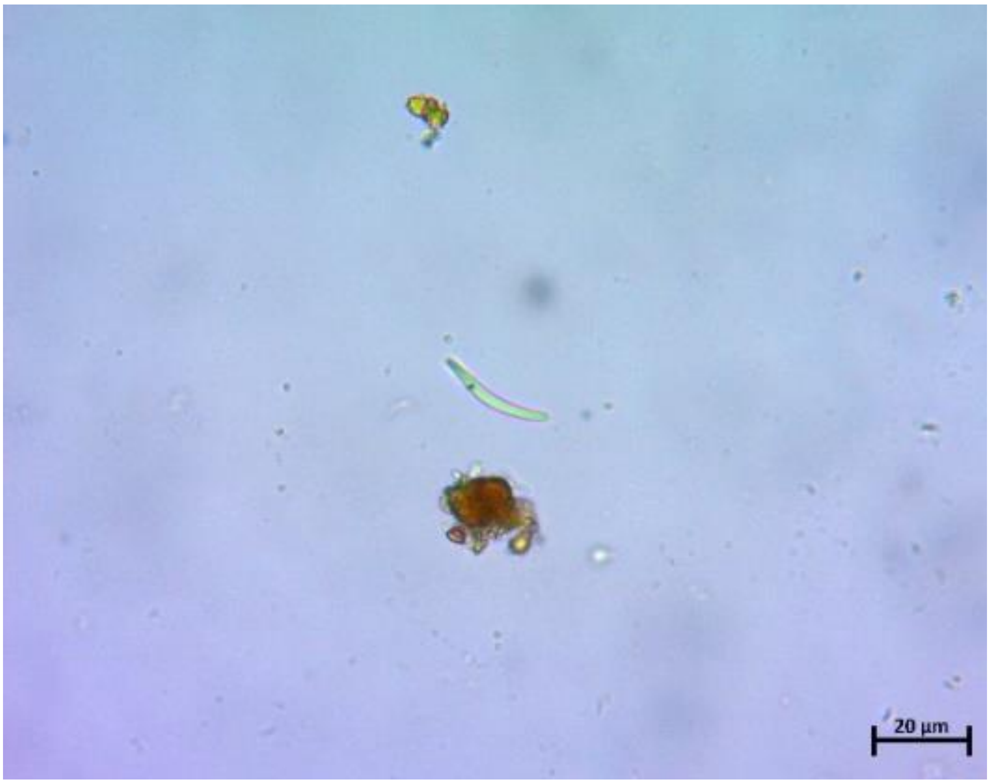

Figure 6. Ascospore of *B. jaapii*

Their measured dimensions approximately match those previously published by various authors (Table 6).

Table 6. Biometric characteristics of ascospores of *B. jaapii* found in naturally infected leaves

Source of information	**Size (µm)**	**Limit values (µm , min-max)**	**Mean value (µm) and standard error**	**Standard deviation**
Own data	length	25.00-45.00	32.8±0.48	4.78
	width	2.50-3.70	3.0±0.05	0.49
Velichkova (1983)	length	24.37-50.62	36.12*	–
	width	5.00-6.87	5.68*	–
Higgins (1914)	length	33.00-50.00	–	–
	width	3.50-4.50	–	–

* Standard error data is missing. ** The data comes from a table that appears in the dissertation's full text.

The description of spring macroconidia (SPM) overlaps with that in the literature. The only difference is that our study's spores are longer (Fig. 7; Table 7).

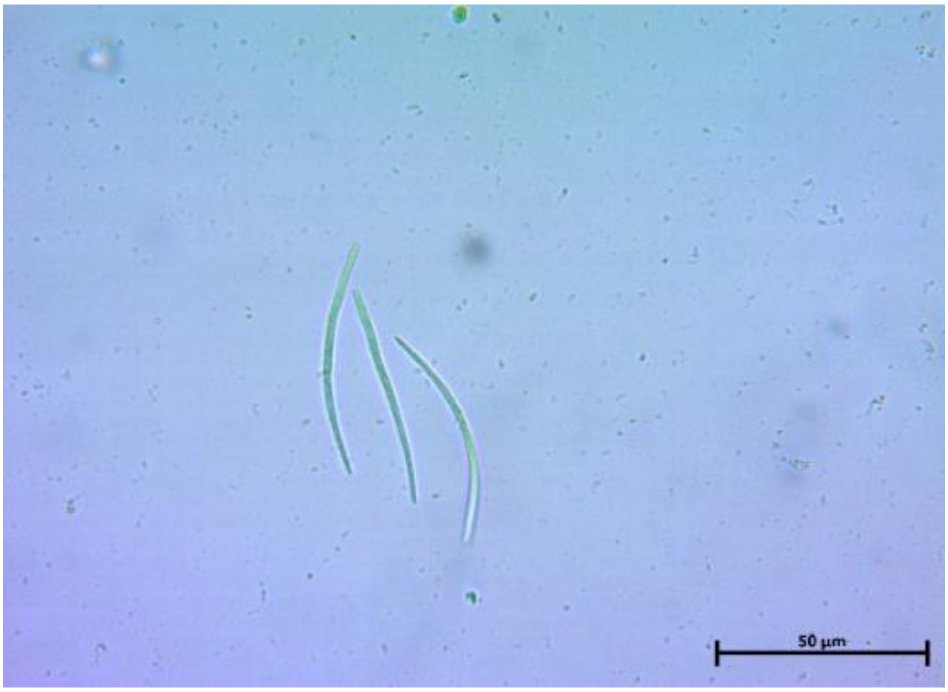

Figure 7. Spring macroconidia of *B. jaapii*, cv. Van, Location: Plovdiv.

Table 7. Biometric characteristics of spring macroconidia of *B. jaapii* found in naturally infected leaves

Source of information	Size (µm)	Limit values (µm, min-max)	Mean value (µm) and standard error	Standard deviation
Own data	length	70.00-112.50	90.50±1.06	10.62
	width	2.50-4.50	2.80±0.05	0.50
Velichkova (1983)	length	37.50-125.00	68.88*	–
	width	3.12-5.62	4.18*	–
Higgins (1914)	length	50.00-80.00	–	–
	width	2.50-4.00	–	–
Darpoux (1945)	length	50.00-80.00	–	–
	width	–	2.00*	–
Stojanovic and Boric (1973)	length	41.60-121.40	79.60*	–
	width	2.96-4.40	2.69*	–
Mijušković (2002)	length	72.60-92.40	–	–
	width	–	3.30*	–
Pedersen et al. (2012) [a]	length	40.00-80.00	–	–
	width	2.00-3.00	–	–
Pedersen et al. (2012) [a]	length	80.00-120.00	–	–
	width	2.00-3.00	–	–

* Standard error data not available; ** Data from Darpoux (1945), Stojanovic and Boric (1973) are cited by Mijušković (2002); [a] SPM with typical size; [b] SPM with non-typical size dimensions.

In this regard, an explanation is given by Pedersen et al. (2012), who, based on the length of the spores, divide SPM into two groups – typical (40-80 µm) and non-typical (80-127.5 µm). Pedersen et al. (2012) describe a maximum length of typical spores that is similar to the minimum value we found in our study. These authors found variation both within a given season and in individual years. The length of this type of spore found by Velichkova (1983) in Bulgaria has a wider range and overlaps with the data in the present study, and the average value is approximately the same.

2. Epidemiology

2.1. Seasonal and diurnal pattern of ascospores and spring macroconidia release

2.1.1. Seasonal pattern of spore release

Data on the pattern of AS and SPM release in *B. jaapii* are limited. Most researchers of this pathogen used glass microscope slides (Velichkova, 1983; Joshua and Mmbaga, 2015; Keitt et al., 1937; Niederleitner and Zinkernagel, 1999; Pedersen et al., 2012), while others used the Rothorod spore sampler (Eisensmith and Jones, 1981b). Spore traps that actively suck air from the environment are standard in studies of airborne pathogens. A classic example is the 7-day Burkard spore trap Manufacturing Co. Ltd., which was selected for the purposes of our study. The specified device makes it possible to precisely track the seasonal and diurnal pattern of AS and SPM release in CLS.

Differences were noted between the individual location/year combinations in terms of the timing of the first spores captured for the season (Table 8). In all years, the first AS was captured between the end of March and mid-April, with a difference of 21 days between the earliest and latest date. The total number of days with AS in the air was between 23 (in Krichim in 2020) and 66 (Plovdiv, 2019). The share of AS in relation to the total number of spores captured for the season (AS+SPM) varied from 46 to 91% in the different years of the study.

In the case of SPM, the first dispersal date was recorded from late March to early May, with a difference of 41 days for the entire study period. The total number of days with SPM presence was between 9 (Botevgrad, 2018) and 36 (Plovdiv, 2019). The share of SPM in the total number of spores (AS+SPM) per season ranged from 9 to 54% in the individual years of the study.

In half of the cases, the green tip stage in the host occurred approximately at the time when the first AS and SPM were caught; in the rest, it occurred approximately 19 days earlier. Despite the apparent synchronous development between the pathogen and the host in some years, there was no risk of infection since the first critical stage had not occurred (leaves older than 6–8 days). The number of spores released at the beginning of the season is relatively small until the first susceptible stage occurs.

Table 8. Characteristics of the seasonal pattern of ascospores and spring macroconidia release of *B. jaapii*

Location/year	Stage "green tip"[a]	Date of first trapped spores		Date of 85% traped spores		Cumulative number of spores m^{-3} h^{-1} ($x10^3$) (85%)		AS:SPM ratio
		AS[b]	SPM[c]	AS	SPM	AS	SPM	
Plovdiv								
2019	20.3.	23.3.	23.3.	23.5.	24.5.	147	15	10:1
2020	15.3.	3.4.	3.4.	22.5.	11.6.	13	10	1:1
Brestnik								
2020	27.3.	2.4.	19.4.	24.5.	24.5.	4	3	1:1
Krichim								
2018	6.4.	13.4.	3.5.	30.6.	2.7.	16	5	3:1
2019	25.3.	28.3.	28.3.	30.5.	20.5.	34	4	9:1
2020	19.3.	3.4.	14.4.	10.6.	10.6.	6	7	1:1
Gabarevo								
2018	2.4.	6.4.	6.4.	26.5.	3.6.	8	4	2:1
Botevgrad								
2018	5.4.	13.4.	3.5.	13.5.	22.5.	6	1	6:1

[a] Date on which ≥50% of the leaf buds of the Van variety (cv. Oblachinska in Botevgrad) are in the “green tip”stage (BBCH 09).; [b] AS – ascospores; [c] SPM – spring macroconidia; [d] 85% of the total quantity for a given location/year.

There are also trends in the date when 85% of the seasons AS are trapped. Most locations reached this threshold in the second half of May (13-26.5). Observations show that this is when the first symptoms usually appear.

Another significant feature of AS is that after the dissipation of their main amount, a reserve of 15% remains, which is depleted in a relatively long period of time. The process takes about a month and is highly dependent on precipitation. An example of a delay is the vegetation in 2018 in Gabarevo and Krichim, where the last amount of AS was released into the atmosphere, respectively, in the first and last ten days of June. A possible explanation for the observed development is the lack of precipitation in May.

The date of capturing 85% of the total SPM varies depending on the location and year. In half of the surveys, similar to AS, this happened in the second half of May, and in the other half the specified threshold was reached in the first ten days of June. An exception was Krichim/2018, where this event occurred on 2.7. Based on the data, the level of 85% SPM in the air as of a certain date almost matches the level in AS in half of the cases (Plovdiv/2019; Brestnik/2020; Krichim/2018 and 2020). Reports indicate a delay of 8 to 20 days in the remaining cases, with Krichim/2019 being an exception. Overall, the majority of both types of spores are exhausted no later than the second half of May. During the period from the beginning of the growing season to the specified period, there is active tree growth and the formation of young and disease-susceptible leaves.

The total number of spores caught for the entire season also differed in the individual location/year combinations (Table 8). The largest amount of both types of primary spores, taken separately, was recorded in Plovdiv/2019. Brestnik/2020 recorded the smallest amount of AS, while Botevgrad/2018 recorded the smallest number of SPM. It is striking that in 2020, the ratio between AS and SPM was approximately the same for all locations. Summary data on the strong variation in the amount of AS and SPM over the years in one location are presented in Table 9.

Table 9. Comparison of the cumulative number of spores (85% of the total for the season) between different years at a given location.

Location	Ascospores (%)	Spring macroconidia (%)
Krichim		
2018	100	100
2019	214	75
2020	38	144
Plovdiv		
2019	100	100
2020	9	62

* The amount of spores in the earliest year for the respective location is taken as 100%, against which the data for subsequent years are compared.

Similarly, information on the number of both types of spores, recorded in one year at different locations, is presented in Table 10. It is evident that in this case too it is difficult to identify a specific relationship, and this is perhaps normal given the differences in the inoculum available in the garden and leaves, respectively. This phenomenon is a result of both natural and subjective human factors.

Table 10. Comparison of cumulative number of spores (85% of total for the season) between different locations in the respective year.

Location	Ascospores (%)	Spring macroconidia (%)
2018		
Krichim	100	100
Gabarevo	54	81
Botevgrad	37	25
2019		
Krichim	100	100
Plovdiv	434	405
2020		
Krichim	100	100
Plovdiv	218	130
Brestnik	68	48

*The amount of spores in Krichim is assumed to be 100% for the respective year, against which the data for the other locations are compared.

Spore trap data show (Fig. 8) that there are typically 3 to 5 stronger dispersal events in AC and SPM per season. Similar information in CLS could guide fungicide treatments during the season's most critical stages.

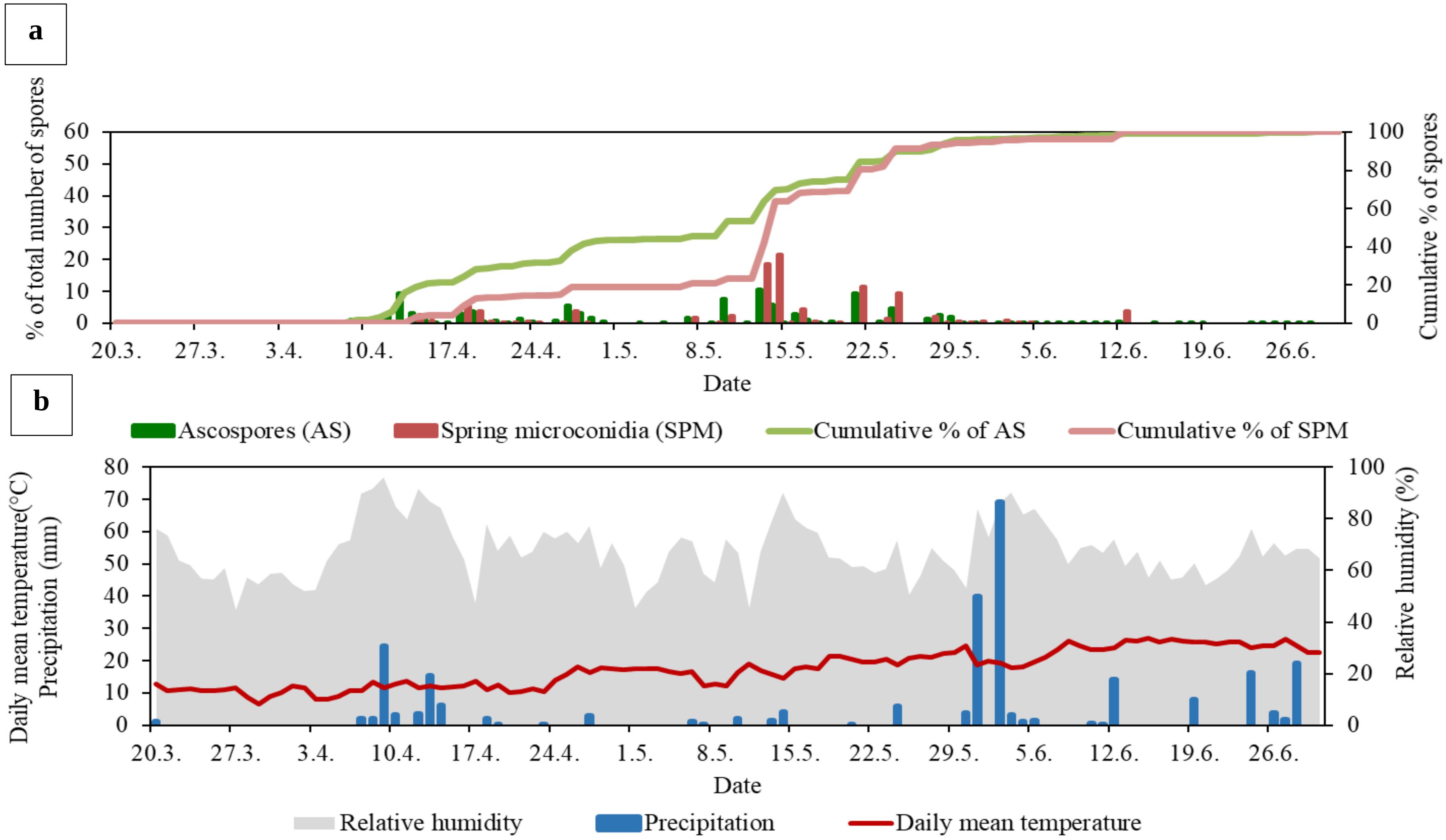

Figure 8. Seasonal spore release of *B. jaapii* (a) and meteorological conditions (b) in Plovdiv, 2019.

The summarized data show (Table 11) that, with the exception of Plovdiv/2019 and Krichim/2018, in the remaining locations, the number of days with AS in the air is greater when there is precipitation compared to those without. In both cases of comparison, even in the absence of precipitation, leaf wetness was recorded. The results show that the release of AS is strongly linked to the presence of precipitation.– a phenomenon also characteristic of other ascomycete fungi. According to some authors (Velichkova, 1983; Keitt et al., 1937), for B. jaapii, a minimal amount of precipitation (0.2 mm) is sufficient to stimulate the process of AS release.

Table 11. Number of days with ascospores under different meteorological conditions

		Number of days with ascospores		
Location	**Year**	**total**	**with precipitation**	**no precipitation**
Plovdiv	2019	66	29	37
	2020	47	31	16
Brestnik	2020	28	24	4
Krichim	2018	54	21	33
	2019	47	28	19
	2020	23	17	6
Gabarevo	2018	37	25	12
Botevgrad	2018	24	18	6

The total number of days with SPM was smaller than that with AS (Table 12), which is probably related to their smaller relative share (average 29%) in the total amount of AS+SPM. Except for Plovdiv/2019, most of the SPM was found on days when it rained, which shows how important this factor is for the type of spores being studied.

Table 12. Number of days with spring macroconidia under different meteorological conditions

Location	Year	Number of days with spring macroconidia		
		total	with precipitation	no precipitation
Plovdiv	2019	36	17	19
	2020	25	25	0
Brestnik	2020	16	15	1
Krichim	2018	18	10	8
	2019	17	11	6
	2020	17	15	2
Gabarevo	2018	21	14	7
Botevgrad	2018	9	8	1

In terms of the total number of days with the presence of one or two types of spores in the air, two types of spores or only AS were simultaneously detected (Table 13).

Table 13. Total number of days with different types of spores in the air regardless of weather conditions

Location	Year	Total number of days		
		With AS / without SPM	AS with SPM	With SPM / without AS
Plovdiv	2019	30	36	0
	2020	22	25	0
Brestnik	2020	13	15	1
Krichim	2018	36	18	0
	2019	30	17	0
	2020	7	16	1
Gabarevo	2018	16	21	0
Botevgrad	2018	15	9	0

The exception is two days during which there was only SPM. The longer survival of AS in the air is largely logical because they are highly specialised structures for transferring the pathogen from one microdistrict to the next.

Since both types of primary spores are strongly dependent on precipitation, it is logical to assume that the trend indicated in the above table also persists when precipitation is available (Table 14).

Table 14. Total number of days with precipitation and different types of spores

		Total number of days with precipitation		
Location	**Year**	**With AS / without SPM**	**SPM + SPM**	**With SPM / without AS**
Plovdiv	2019	12	17	0
	2020	6	25	0
Brestnik	2020	9	15	0
Krichim	2018	11	10	0
	2019	17	11	0
	2020	3	14	1
Gabarevo	2018	11	14	0
Botevgrad	2018	10	8	0

Of interest are the summarised results for the types of spores when there was no precipitation during the day (Table 15). Here, cases where only AS was present are categorically predominant, there are fewer cases with two types of spores present, and there are practically no established cases with only SPM. Such a result shows that with available leaf wetness and appropriate temperature, even if there is no precipitation, it is possible for infections to occur. The possibility of this happening with ascospores is much more real given their longer-term presence in the air.

Table 15. Total number of days without precipitation and different types of spores

Location	Year	Total number of days without precipitation		
		With AS / without SPM	AS + SPM	With SPM / without AS
Plovdiv	2019	18	19	0
	2020	16	0	0
Brestnik	2020	4	0	1
Krichim	2018	25	8	0
	2019	13	6	0
	2020	4	2	0
Gabarevo	2018	5	7	0
Botevgrad	2018	5	1	0

The results of the analysis of 31 events show that in most cases AS and SPM begin to disperse at the hour when it starts to rain (Fig. 9). A smaller but significant share falls on launches that begin one or two hours after the indicated moment. The lowest share of first AS captured is four to five hours after the start of precipitation. The start of AS launch with a delay in relation to the precipitation event can be explained by the need for additional maturation and moistening.

In SPM, a trend similar to that described in AS is observed, but with the difference that 6 hours after the onset of precipitation, no initial spore dispersal is recorded. This may be due to a directly precipitation-dependent mechanism of release or slow maturation of SPM.

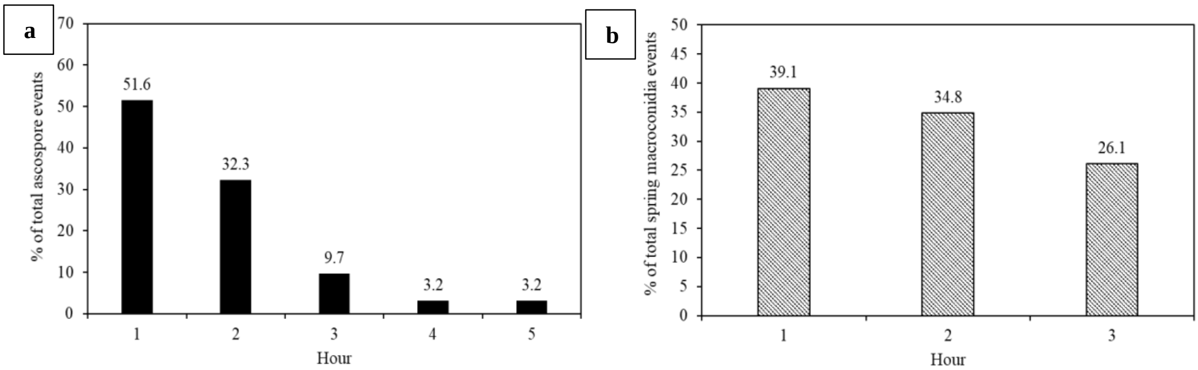

Figure 9. Share of the total number of events with first AS (a) and SPM (b) released at a certain hour (the 1st hour coincides with the start time of precipitation).

The data were also analysed in terms of the number of primary spores released after a rainfall event (Fig. 10). The largest number of AS released was found in the interval from 1 to 13 hours after rainfall. During rainfall and after 13 hours, the amount of spores released is about 3-10 times less.

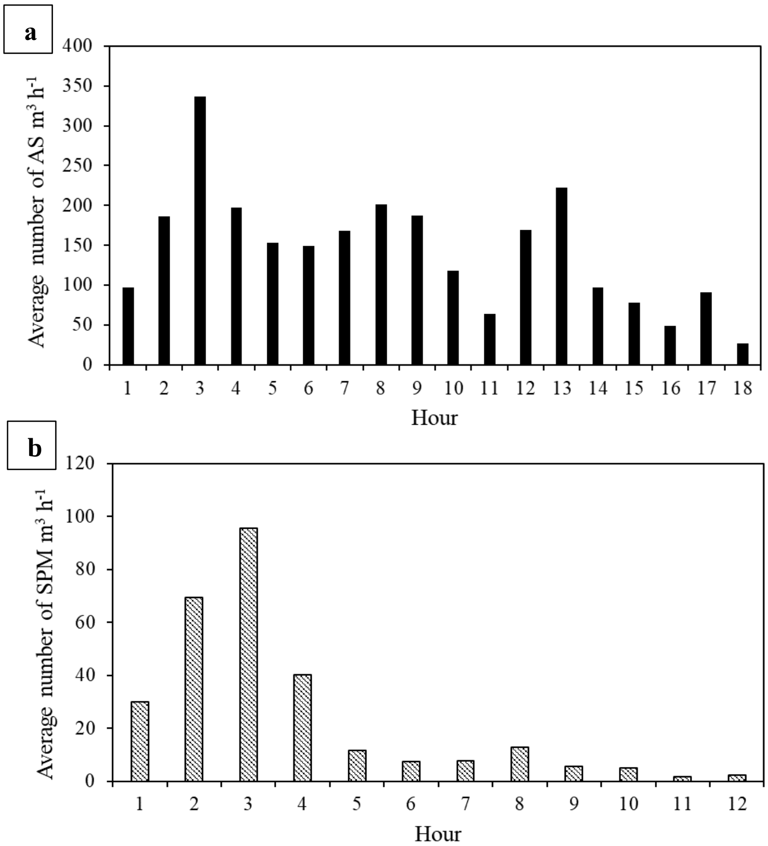

Figure 10. Average number of AS (a) and SPM (b) for a given hour after the start of precipitation (the 1st hour coincides with the start time of precipitation).

The trend for SPM was similar, with the difference that their amount sharply decreases 5 hours after precipitation. From the studied sample, it can be summarized that with the same precipitation, the amount of AS dispersed in the air is more than SPM. In some cases, the amount of SPM is higher than AS.

Predictive models could incorporate information on the period and quantity of AS and SPM released. Farmers' access to such online information would help for more precise application of fungicides in the so-called "window" of germination of the spores that have not yet infected. Such practice has a leading importance in the control of apple scab in integrated and organic production.

2.1.2. Diurnal pattern of spore release

The dispersal of spores within a day varies in different locations and years. Multi-year data (Fig. 11) show that in the AS the amount is more significant in the interval 01:00-07:00 h, while in the SPM between 14:00-16:00 h. The results in the individual location/year combinations show differences.

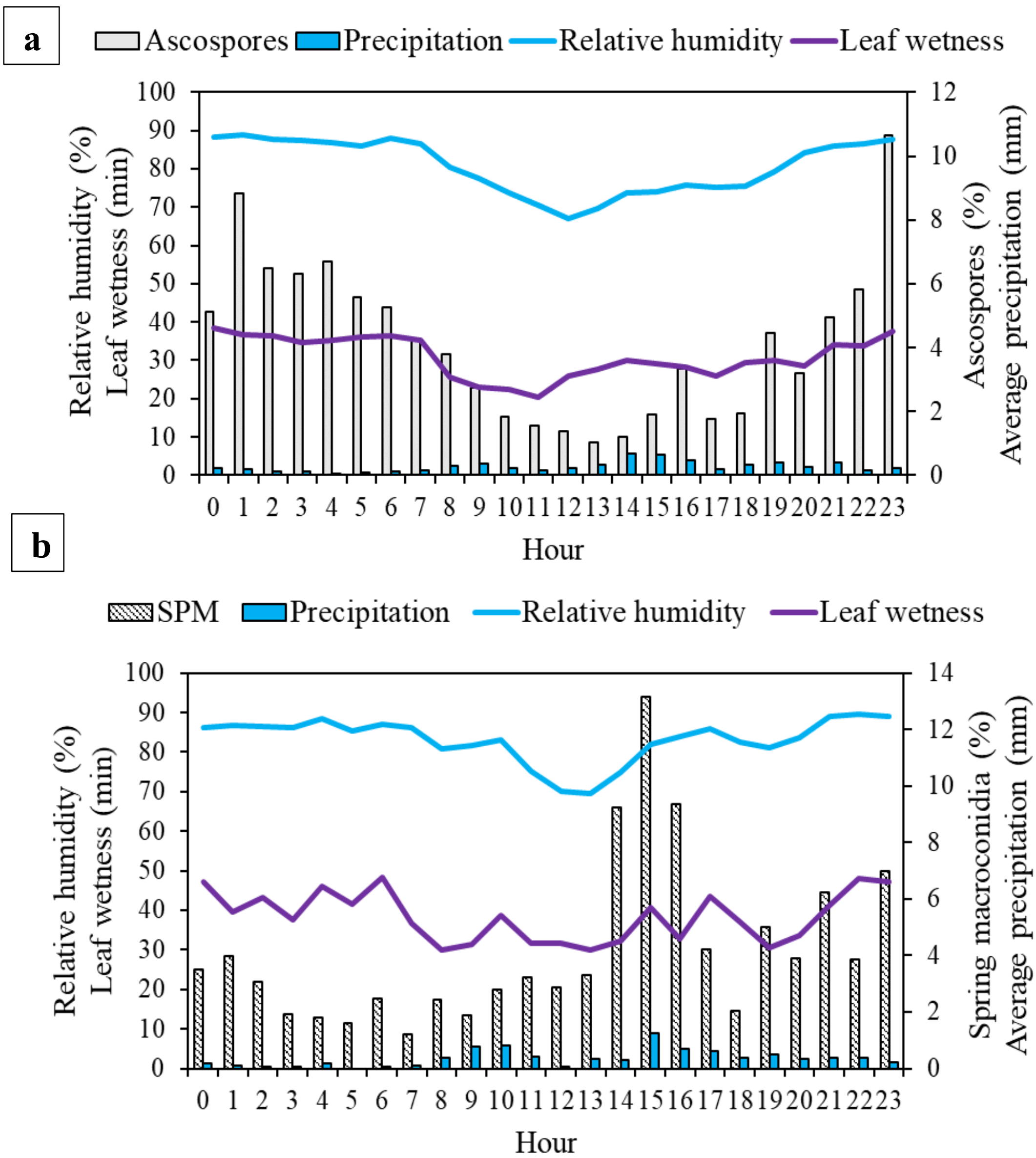

Figure 11. 24-hour dynamics of AS (a) and SPM (b) and meteorological conditions (average for the period 2018-2020).

The data from the present work, similar to observations in the USA (Keitt et al., 1937), confirm that the dispersion of AS is independent of light. This phenomenon likely stems from the pathogen's unitunicate (single-layered) asci. In comparison, in ascomycete fungi with bitunicate asci, such as *V.* inaequalis, the release is mainly during the day.

As already mentioned above, the development of SPM is more strongly dependent on precipitation compared to the development of AS, and this is clearly visible in Figure 12 and Figure 13. On days without precipitation but with the presence of leaf wetness (Fig. 12) there are extremely few SPM, and this is in an atypical period for them, such as the beginning of the night. Therefore, these are probably spores left over from previous events that have been released from the fruiting bodies. AS are significantly higher and at a typical time for them (night and early morning), which is an indication that with leaf wetness, there could be a discharge. The graph excludes the hours outside its range due to the absence of leaf wetness.

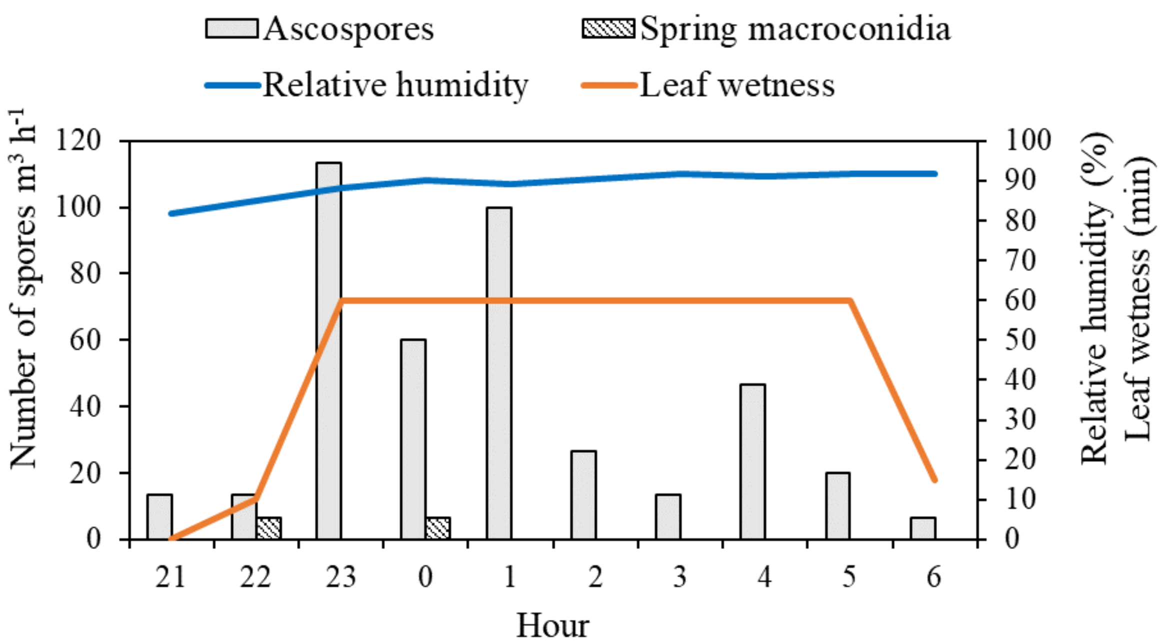

Figure 12. Spore dispersal in the presence of leaf wetness. 7.5.-8.5.2018; Location: Botevgrad.

No less intriguing is the information collected regarding the types of spores in the air in the absence of leaf wetness or precipitation (Fig. 13). SPM is completely absent, and the picture in the case of AS in terms of time interval and quantity is analogous to that in the presence of leaf wetness. In this scenario, the question emerges about the release of ascospores in the absence of precipitation and leaf wetness.

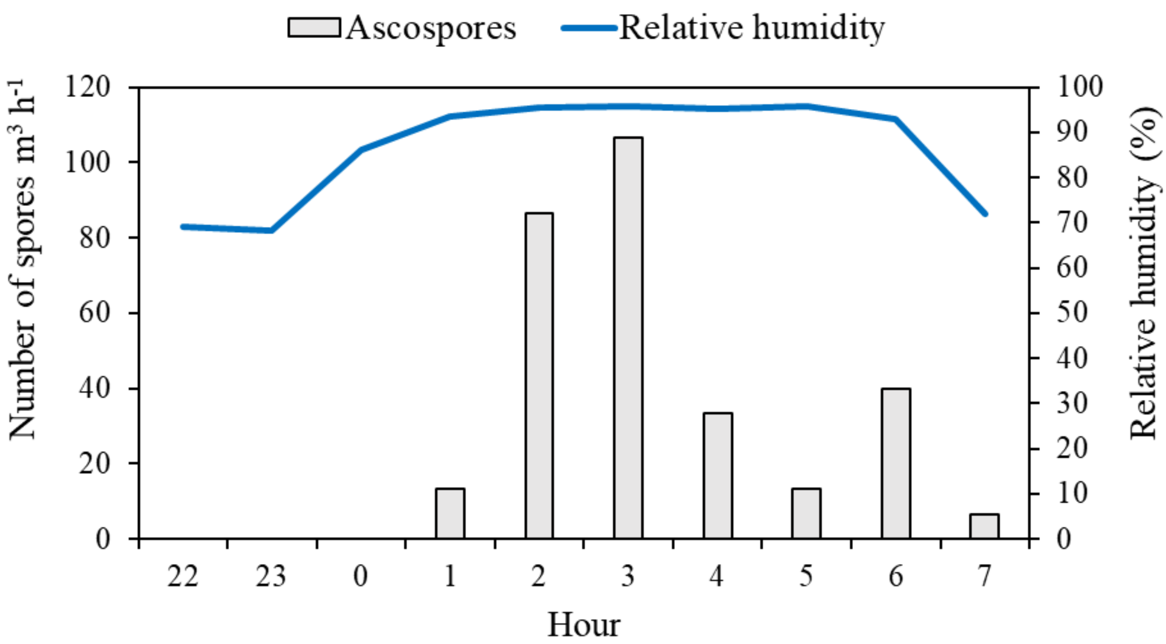

Figure 13. Spore dispersal in the absence of leaf wetness. 19.5.-20.6.2018; Location: Krichim.

The summarised results show that AS are the main source of primary inoculum in Bulgaria. SPM also has a significant share in certain years and contributes to increasing infectious pressure. This is indicated by 2020, when SPM is equal to or greater than AS in each experiment location. The availability of information on the dynamics of spores present in the air is of key importance for determining the real risk of infection.

2.2. Development of apothecia and spring acervuli under laboratory conditions

Under the same conditions, the current results show that the maturation of AS and SPM takes about the same amount of time, 311 and 331 GD (Fig. 14). In apothecia, the initial development up to 93 GD is very slow, followed by a sharp acceleration of the process up to 160 and a slight slowdown up to 311 GD. The pattern of SPM maturation is very similar to what was reported before, with one small difference: they mature faster up to 66 GD, and then the process slows down for a short time between 66 and 133 GD before speeding up again. It is notable that the curve in both types of spores has a sigmoid shape, which is typical for other ascomycete fungi – *V. inaequalis* and *V. pirina* (Eikemo et al., 2011; Gadoury and MacHardy, 1982a).

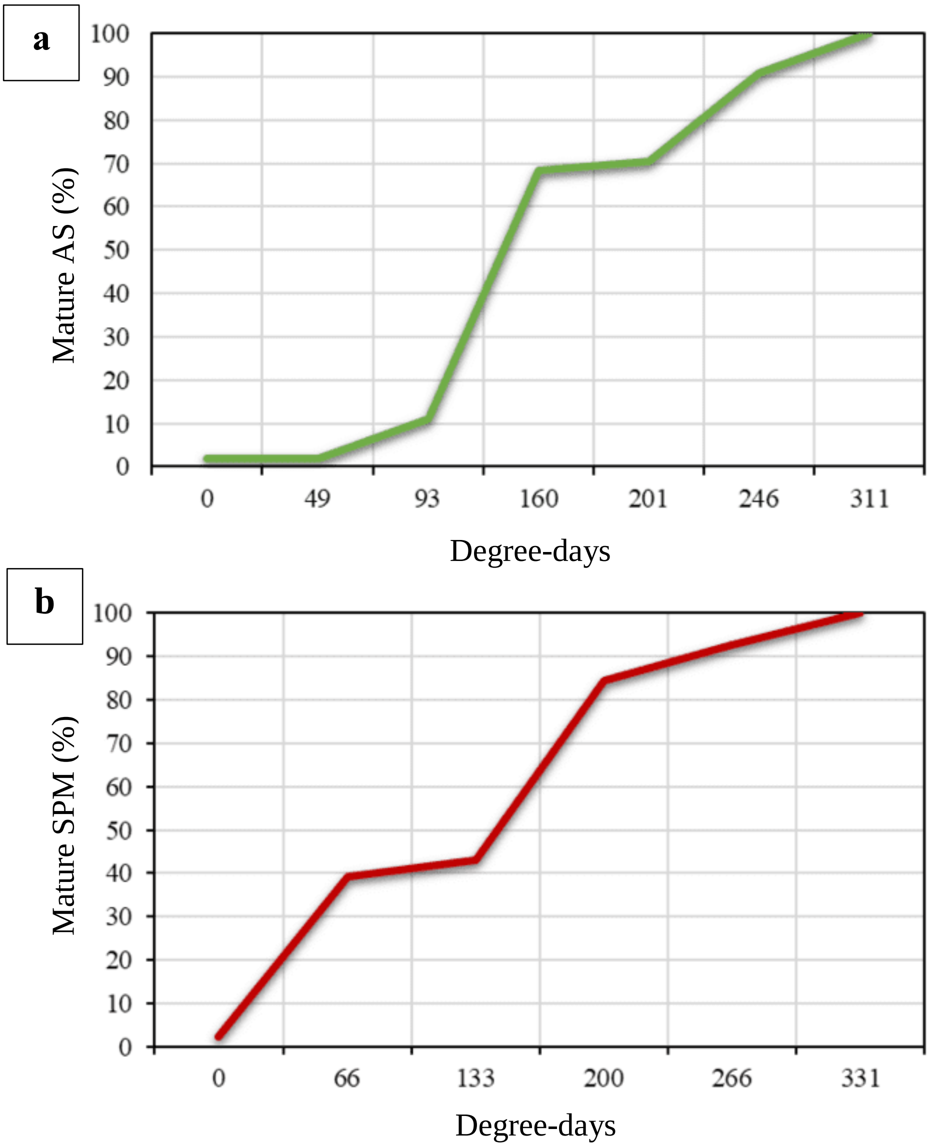

Figure 14. Cumulative proportion of mature AS (a) SPM (b) relative to degree days (leaves).

The depletion of the ascospore stock for 311 DG at 22°C means that the maturation process under controlled conditions is approximately 14 days at a temperature base of 0°C. Data on the conditions required for AS maturation in *B. jaapii* are limited, and for SPM, according to world literature, they are completely absent.

2.3. Dynamics of leaf litter decomposition after leaf fall

There is still a lack of sufficient data on the decomposition of cherry and sour cherry leaves under various weather conditions and growth systems. A more detailed study on this topic was carried out in Hungary by Holb (2013). Multi-year data from such experiments would serve to create a predictive model for the potential primary infection in CLS. The data for the study are presented in Table 16.

Table 1 6. Proportion of soil surface covered with leaves

	Soil surface covered with leaves (%)								
Location/year	**30.11.**	**30.12.**	**30.01.**	**28.02.**	**30.03.**	**30.04.**	**30.05.**	**30.06.**	**15.07.**
Plovdiv									
2018/19	100	100	99	95	90	70	40	10	5
2019/20	100	100	100	95	80	65	45	15	10
Brestnik									
2019/20	100	100	100	90	80	60	40	20	10
Krichim									
2017/18	100	100	100	90	80	75	55	35	10
2018/19	100	100	99	95	90	80	50	10	5
2019/20	100	100	100	90	80	60	45	15	5
Gabarevo									
2017/18	100	100	99	90	80	75	50	30	15
Botevgrad									
2017/18	100	100	100	90	75	60	45	20	10

* In the study area, 100% of the soil surface was covered with leaves at the beginning of the experiment.

The survey can be described in general terms in three stages. The first covers the period 30.11-30.03, the second 01.04-30.05 and the third 01.06-15.07. The summarised data for all location/year combinations show a reduction in leaf mass during the first stage from 10 to 25% with an average value of the indicator of 18.1%. During the second period, the reduction is 30-50%, with an average value of 35.6%, and during the third, between 30-45% and an average of 37.5%. The final amount of retained leaf mass, measured on 15.07 for all considered cases, does not differ significantly and is in the range of 5-15%.

2.4. Development of CLS during the season

2.4.1. Disease monitoring

The monitoring of the CLS covered locations in three regions of the country for a period of three years. The collected data allowed for the establishment of certain relationships between the symptoms, phenological stage of the plant, and meteorological factors (Table 17).

Table 17. Development of the CLS during the period 2018–2020

		First appearance of			Date on which the value is > 20%	
Location	**Year**	**ascospores**	**symptoms**	**defoliation**	**incidence**	**defoliation**
Plovdiv	2018	Apr 17	June 16	July 21	July 14	July 28
	2019	Apr 14	May 3	June 21	May 26	Sep 1
	2020	Apr 5	May 9	July 28	July 14	lower
Krichim	2018	Apr 20	June 1	June 22	June 30	July 7
	2019	May 6	May 18	June 22	June 16	July 14
	2020	Apr 22	May 16	July 28	Aug 4	lower
Gabarevo	2018	May 8	May 21	June 18	July 14	July 7
	2019	Apr 22	May 21	June 18	June 16	July 7
Botevgrad	2018	Apr 25	May 19	June 9	June 2	June 23
Brestnik	2020	Apr 23	May 16	July 28	lower	lower

The dates when the disease incidence and defoliation reached the 20% threshold receive special attention. A review of the graphs (Fig. 15) shows a strong similarity in the development of the disease in three locations (Plovdiv, Krichim, and Gabarevo) and two neighboring districts in southern Bulgaria in 2018.

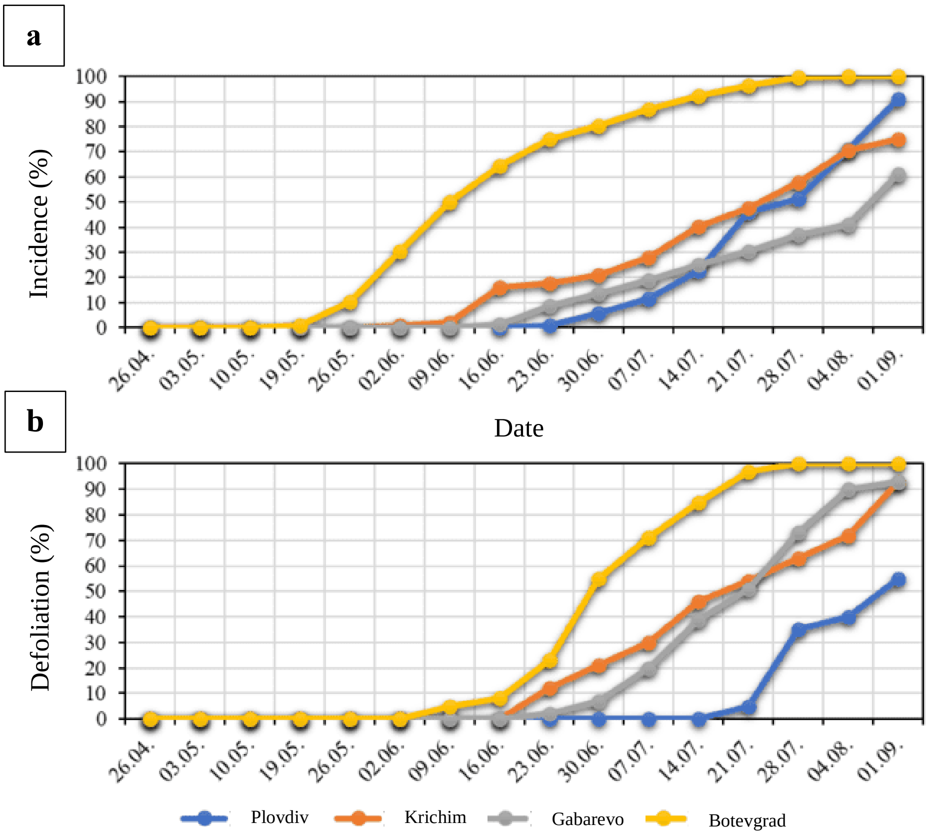

Figure 15. Incidence (a) and defoliation (b) by CLS, 2018

An exception is the development of CLS in Northern Bulgaria, and more precisely Botevgrad, where the disease appeared earlier than in the other three locations and quickly reached 20% prevalence (2.6.) and 20% defoliation (26.6.). The disease infected all available leaves in late July and early August, followed shortly by complete defoliation in the same orchard. In early September, the trees in the other orchards were also defoliated as a result of the CLS infection.

From the monthly meteorological data (Fig. 16) it is evident that the amount of precipitation is directly related to the spread of the pathogen in the plantation. The air temperature is within the optimum and is almost constant for the different locations. Throughout the season, the distribution of precipitation was relatively uniform, with some exceptions.

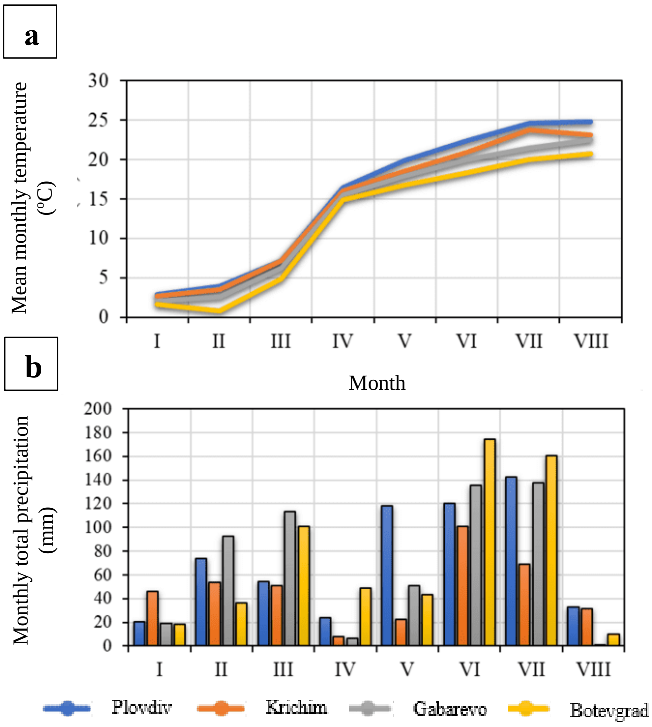

Figure 16. Temperature (a) and precipitation (b) during the period January-August, 2018.

The month with the least precipitation was April; specifically, in Botevgrad, the amount was 48.6 mm; in Plovdiv, it was twice as low (23.8 mm), in Krichim and Gabarevo, it was insignificant (approx. 7 mm). In May, precipitation was on average about 50 mm. In these two months, they were not very favorable, and this led to a delayed initial development in June. In the period June–July, there was a well-expressed precipitation situation, with monthly values of the indicator ranging from 70 to 174.4 mm. As a result, a progressive spread of the disease followed, including defoliation from the end of June to September. The optimal distribution of precipitation throughout the season is notable, including the presence of very high amount in Botevgrad.

The development of the disease in 2019 was similar to that in the previous year (Fig. 17; Fig. 18), with gradual CLS development throughout the season from May to September. Compared to 2018, April was rainier in 2019 and influenced the earlier start of the epidemic. Several important points stand out from the same graphs. The spread of the disease correlates very well with defoliation in Krichim and Gabarevo, and in Plovdiv it slows down in both years.

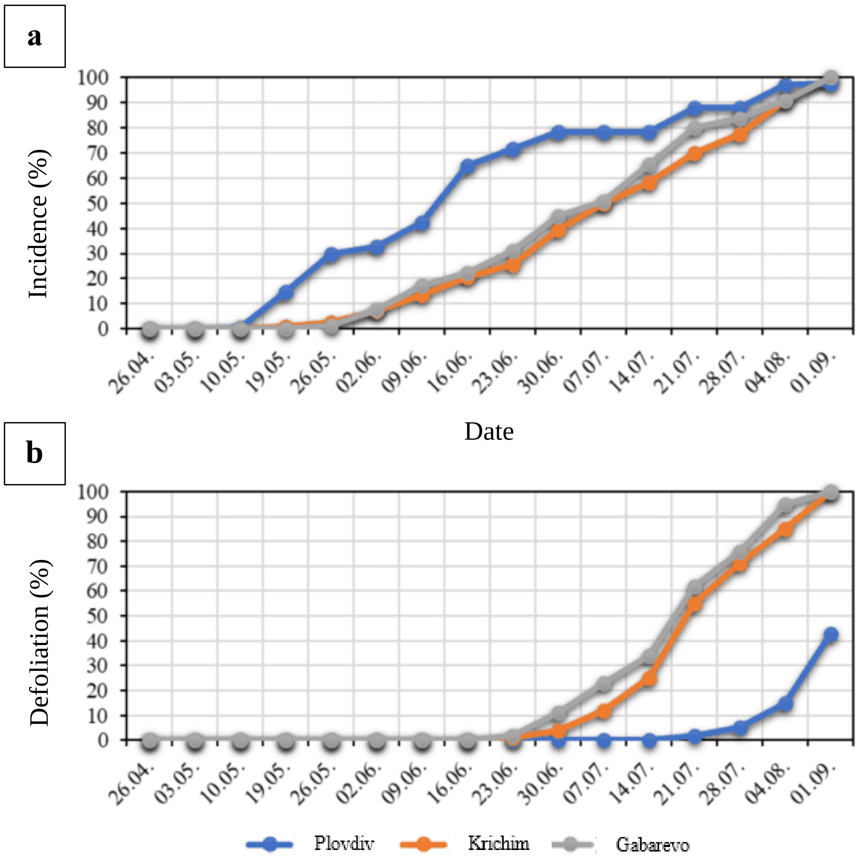

Figure 17. Distribution (a) and defoliation (b) by CLS, 2019

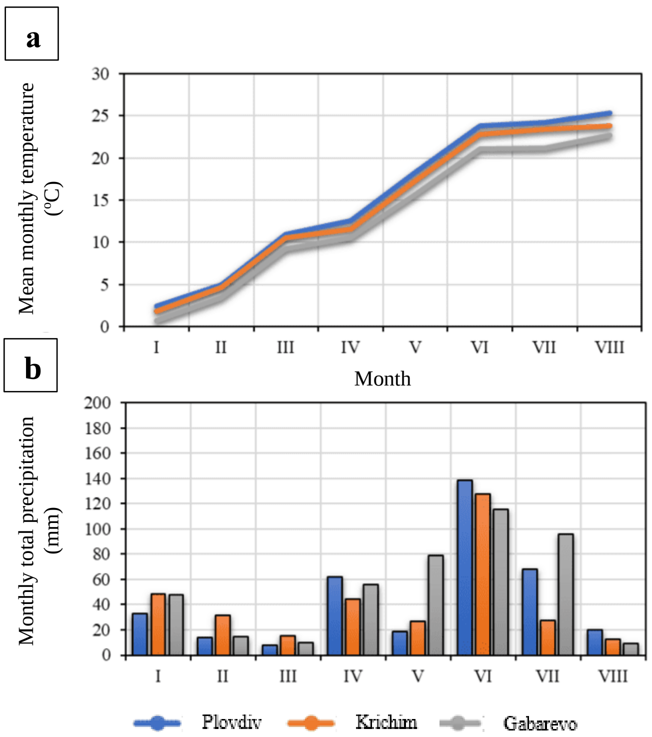

Figure 18. Temperature (a) and precipitation (b) during the period January-August, 2019.

Although the weather was similar in 2018 and 2019 and the development curves were not the same, we believe that the buildup of inoculum from 2018 played a part in the stronger attack by the CLS in 2019.

The progression of CLS in the last year of the survey (2020) can be categorized as weak or medium (Fig. 19). The disease appears within the normal range (end of May and beginning of June) and by the end of July maintains levels no higher than 10 and 20% infected leaves. These values slightly increase by the beginning of September for Plovdiv and Krichim and are lower in Brestnik.

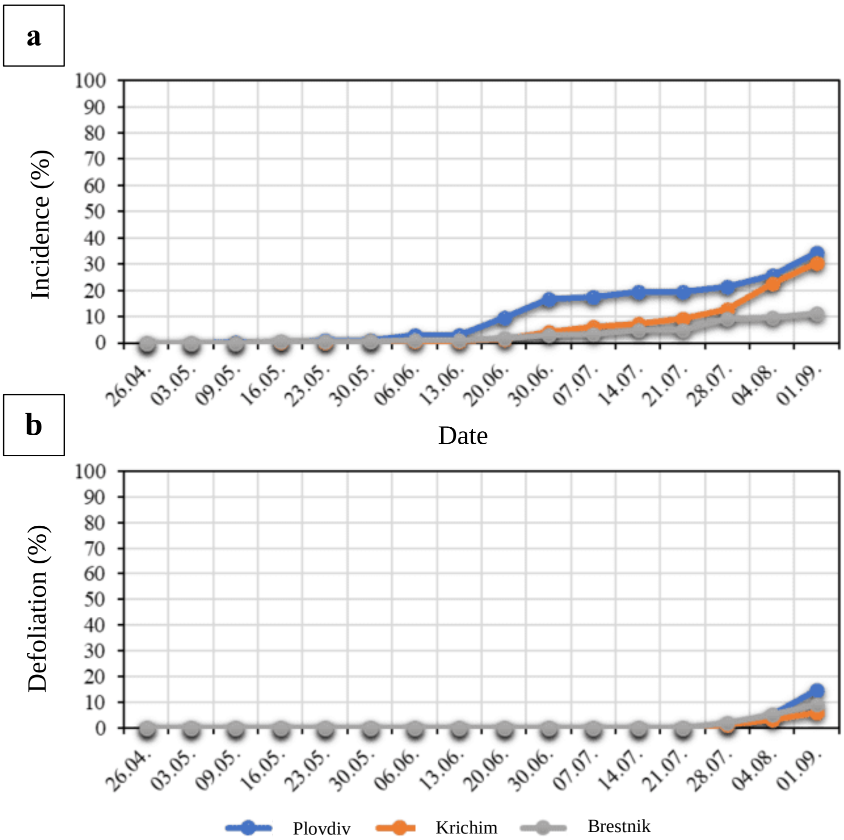

Figure 19. Incidence (a) and defoliation (b) by CLS in 2020.

The average monthly values of air temperature and precipitation for the three locations almost coincide, with the exception of Krichim, where precipitation in May was 2.5 times lower than in the other two locations (Fig. 20).

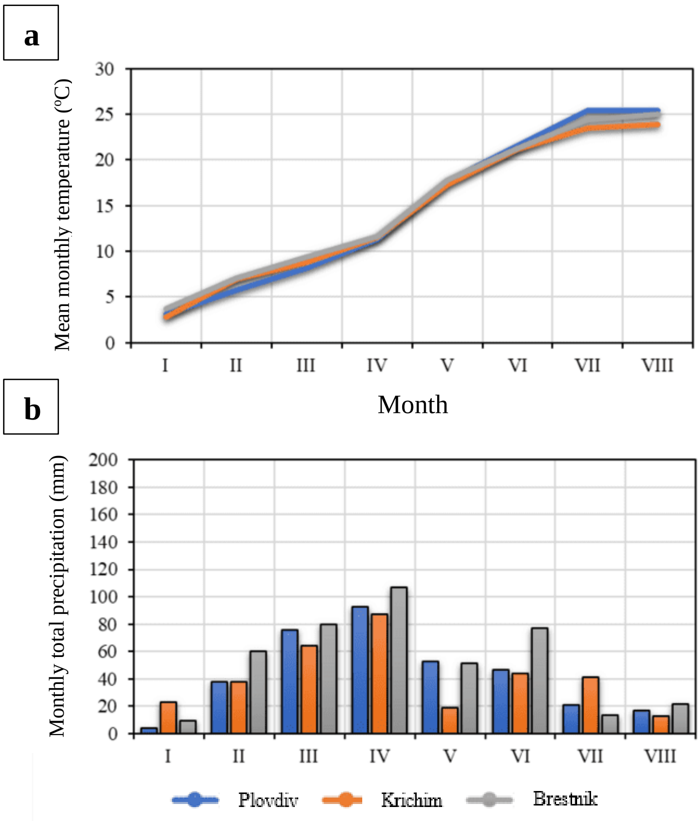

Figure 20. Temperature (a) and precipitation (b) during the period January-August, 2020.

It is noteworthy that precipitation was more stable in locations and significant in quantity during the months of March and April, after which it became more erratic and reduced in the rest of the season. During the period July–September–August, it was absent or negligibly low in value. Regarding the locations of Krichim and Plovdiv, data exist for all three seasons, which allows some conclusions to be drawn. In the period 2018-19, the conditions for the disease were generally favorable, with the only difference being that in 2018, there was a certain delay in the initial appearance of symptoms, and this was explained by a possible low amount of inoculum than in the previous season. The general trend in terms of meteorological factors is that in April and May, precipitation was generally uneven in the locations, but in June and July significant in quantity.

In 2020, the situation is diametrically opposite; optimal precipitation was in March and April, after which it decreased in the months until the end of the season. The described scenario leads to limited spread of the disease in the last year in all three locations. The infectious background factor should be ignored, since in the fall of 2019 its level was high. The months of June and, to some extent, July stand out with a key role in the epidemiological development of the disease. The amount of precipitation in April is two to five times greater than in 2020, but this did not significantly affect subsequent events. The occurrence of a secondary infections in June ensures rapid development of CLS via summer macroconidia. Temperatures are optimal for infection and are not yet extremely high in June, which is related to the longer retention of leaf wetness on the leaves after precipitation.

2.4.2. Influence of meteorological factors on the development of CLS

The importance of meteorological factors for the development of the CLS disease suggests the application of additional and more in-depth analyses using different statistical methods and approaches. The use of panel analysis is based on some of its advantages. Panel data analysis aims to detect unobserved heterogeneity, both between studied indicators and in the time period, since this heterogeneity cannot be detected by time series analysis or by cross-section (Eom et al., 2008).

In the present study, meteorological factors (average temperature, precipitation, leaf wetness) were calculated according to the indicated methods for the respective location. In general, heterogeneity was observed in terms of the number of infected leaves per location. Certain periods in Krichim and Plovdiv reported homogeneity, likely due to their close geographical proximity and similar meteorological conditions. It is noteworthy that in Krichim, Gabarevo, and Plovdiv, a large part of the leaves were infected in the temperature range of 20-25℃, and in Botevgrad at 15-20℃. This is somewhat understandable, considering the higher altitude and lower temperatures in the Botevgrad region.

In all locations, the majority of infected leaves were recorded at rainfalls from 0 to 30 mm. In terms of leaf wetness, the majority of infected leaves were found in the range of this parameter 0-50 h. The exception is the garden in Botevgrad, where, probably due to more frequent rainfall and high relative humidity, they were in the interval 60-100 h. It is possible that the specificity for each location (α_i)may be related to the independent variables such as meteorological factors. The results of the preliminary tests of the fixed effects model showed high significance.

Due to the presence of heteroscedasticity and autocorrelation in the residual component, cluster standard errors using the Arellano method were used to neutralize the effects of the above two problems (Wooldridge, 2013), which made the standard formulas invalid (Table 18).

Table 18. Two-way fixed effects with clustered standard errors used

Variable	**Rating**	**Standard error**	**Statistics**	**p-value**	**Level of significance**
Average temperature	0.685	0.167	4.104	0.000	***
Precipitation	0.026	0.011	2.400	0.017	*
Leaf wetness	0.005	0.013	0.408	0.683	
Adj R-squared [a]	-0.05	R2	0.0132		
F-statistic	6.037	p-value	0.0004		
Degrees of freedom	3; 1350				

[a] Adj R-squared – Adjusted r-squared

Statistically significant values at the level of agreement $\alpha = 0.05$have the regression coefficients of average temperature and precipitation amount, both coefficients are positive and show a positive relationship and causation with the dependent variable. With an increase of 1°C on average for the previous 11 days, the number of infected leaves is expected to increase by 0.69%, with other variables fixed. The effect of precipitation is less significant and shows that with an increase in the amount of precipitation by an average of 10 mm for a seven-day period, an increase of 0.26% of infected leaves is expected after a week. The effect of leaf wetness is not statistically significant.

In the calculation of the two-way fixed effects model with three interactions, double centering was performed to achieve the effect of the within-group estimator (the effect of the within-group variation). The standard errors were again corrected with clustering by the Arellano method to control for heteroscedasticity and serial autocorrelation (Table 19).

Table 19. Two-way fixed effects with triple interaction and cluster standard errors used

Variable	Rating	SE [a]	Statistics	p-value	Level of significance
Average temperature	1.2831	0.2534	5.0634	0.0000	***
Precipitation	-0.0029	0.0109	-0.2693	0.7877	
Leaf wetness	0.0633	0.0200	3.1677	0.0016	***
Avg. temperature:Precipitation	0.0637	0.0103	6.1689	0.0000	***
Avg. temp.:Leaf wetness	-0.0208	0.0039	-5.3983	0.0000	***
Precipitation:Leaf wetness	0.0012	0.0005	2.3426	0.0193	**
Avg. temp.*Precipitation*Leaf wetness	-0.0021	0.0004	-5.0600	0.0000	***
Total sum of squares	37279	R2	0.08803	F-stat.	18.5608
Residual sum of squares	33998	Adj. R^2	0.025018	p-value	< 2.22e-16
ID	40	T	16-48	N	1440

[a] SE – standard error

Substituting the coefficients from the panel regression yields the following equation (1):

$$\ddot{y_{it}} = 1.283\ Avg.temp. - 0.003\ Prec. + 0.0633\ LW + 0.0637\ Avg.temp.*\text{Валеж} - 0.0298\ Avg.temp.*LW + 0.0012\ Prec.*LW - 0.002\ Avg.temp*Prec.*LW + v_{it}, \quad (1)$$

Statistically significant at the level of agreement $\alpha = 0.05$. The explanatory power of the model is still low, but the smoothed coefficient of determination is already positive and is approximately 3%. For interpretation of the results, the expression of Equation 1 is divided into two parts. The first part represents coefficients not including the average temperature, which are constants of the model.

The second part represents the slope of the linear relationship and includes all coefficients where the average temperature (2) is involved:

$$\ddot{y}_{it} = (-0.003Prec. + 0.0633LW + 0.0012Prec. * LW) + Avg.temp.(1.283 + 0.0637Prec. - 0.0201 * LW - 0.0021Prec. * LW) + v_{it}, \quad (2)$$

The mean temperature is the main independent variable that can describe leaf infection through the moderator function (Jaccard et al., 2003). The slope is calculated using the regression coefficients from the second part of the model (Equation 8), as well as additional coefficients created for high and low values of the independent variables precipitation (mm) and leaf wetness (h). The regression coefficients from the first part of Equation 8 are used to calculate the model constant. The values are calculated based on the sum/difference (high/low value) of the arithmetic mean for the variables and their standard deviation (Table 20).

Table 20. Descriptive statistics of the data for the studied locations

Variable	**Number of units**	**Arithmetic mean**	**SD [a]**	**Min.**	**25%**	**Median**	**75%**	**Max.**
Location	1,440	2.889	0.994	1	2	3	4	4
Tree	1,440	5.5	2.873	1	3	5.5	8	10
Infected leaves, cumulative	1,440	28.38	34.135	0	0	11.81	52.75	100
Infected leaves	1,440	4.828	6.413	0	0	2	7.816	41,247
Average temperature	1,440	20,216	3.593	11,282	17.7	20,495	23,382	25.945
Precipitation	1,440	16,398	22,428	0	1.375	8.45	19.1	114.7
Leaf wetness	1,440	32,768	21,246	0	17.25	29,417	46.125	100,083

[a] SD – standard deviation

Four cases of slope and constant are obtained, depending on the combination of precipitation and leaf wetness values (Jaccard et al., 2003). The four slopes reflect the partial effect of the triple interaction on infected leaves in the two-way fixed effects model (Fig. 21). The two slopes of the combinations of low precipitation and high leaf moisture and high precipitation and low leaf moisture are close to each other and show that there is a linear relationship between average temperature and infected leaves. The relationship is positive, with a gentle slope, i.e., a small coefficient. An inverse (negative) relationship is observed between average temperature and the number of infected leaves at high precipitation and high leaf wetness.

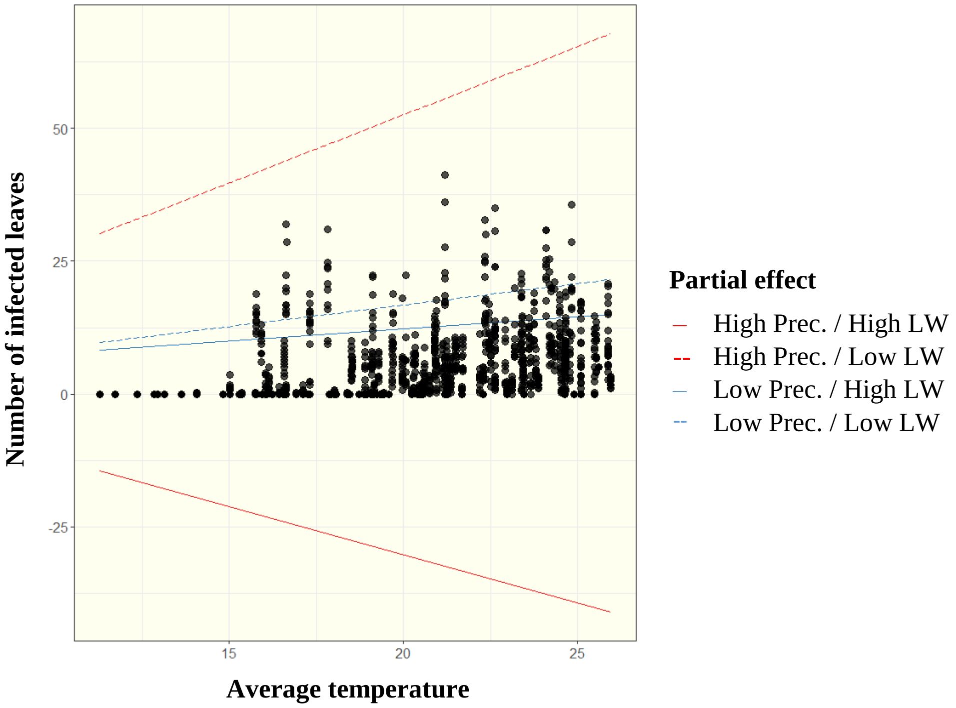

Figure 21. Slope of the partial effect of the triple interaction on infected leaves in the two-way fixed effects model.

The interaction effect with temperature shows that at high temperatures, high amounts of precipitation and leaf wetness, and a smaller number of infected leaves on trees are expected. The last combination of high precipitation value and low leaf wetness again interacts with temperature in a positive influence on the number of infected leaves. The slope of the linear relationship is significantly steeper, and the correlation coefficient between average temperature and infected leaves is – 1.283, on average for the model, in the absence of interaction and fixed values of the other two variables. This means that a greater amount of precipitation, a shorter duration of leaf wetness, and a high temperature will affect the infection more significantly, and more infected leaves are expected.

Due to the need to equalize the scales of the variables (Bring, 1994), the standardized coefficients of the model with two-way fixed effects and triple interactions are also presented (Table 21).

Table 21. Two-way fixed effects model with triple interaction for standardized variables

Variable	**Rating**	**SE [a]**	**Statistics**	**p-value**	**Level of significance**
Average temperature	0.7049	0.1392	5.0634	0.0000	***
Precipitation	-0.0102	0.0379	-0.2693	0.7877	
Leaf moisture	0.1865	0.0589	3.1677	0.0016	**
Average temperature:Precipitation	0.7744	0.1255	6.1689	0.0000	***
Avg. temp.:Leaf wetness	-0.2149	0.0398	-5.3983	0.0000	***
Precipitation: Leaf wetness	0.0795	0.0339	2.3426	0.0193	*
Avg. temp.:Precipitation: Leaf wetness	-0.4828	0.0954	-5.0600	0.0000	***
Total sum of squares	918.4	R	0.08803	F-stat	18.5608
Residual sum of squares	837.55	Adj. R	0.025018	p-value	< 2.22e-16
ID	40	T	16-48	N	1440

[and] SE (standard error) – standard error

The results confirm the direction of the dependencies, only distinguishing the double interaction between average temperature and precipitation as the dependence with the strongest coefficient, followed by that of average temperature and the triple interaction. The analysis of panel data for the studied locations shows a strong positive dependence between average air temperature and a less significant one for the other variables regarding the incidence of CLS on the leaves. The application of a triple interaction between average air temperature, sum of precipitation, and leaf wetness has an effect on the direction of the studied dependence. Large amounts of precipitation combined with a low value of leaf wetness contribute to enhancing the effect of increasing temperature on the appearance of new infected leaves. Prolonged leaf wetness in combination with a large amount of precipitation shifts the dependence between high temperature and the number of infected leaves in a negative direction. In this case, the analysis shows that at lower temperatures in the sample, a greater number of infected leaves is expected.

Some features of the experiment protocol should be considered when interpreting the data. According to the results, temperature stands out as the main factor for the appearance of symptoms on the leaves. The forecast is made based on an 11-day period, which ends 3 days before the reporting date and best describes the incubation period of the pathogen. This likely explains why temperature has a greater impact than precipitation. In the presence of precipitation, if the temperature is high, the leaf wetness should also be shorter in duration because it evaporates faster.

2.5. Validation of a predictive model using potted trap plants

The results of the two-year experiment show that the number and duration of the periods in which the plants were exposed to a natural infectious background were similar. Using the Eisensmith and Jones model, 32 infectious periods were recorded in 2018. The shortest of them lasted 1 hour, and the longest—27 hours. The average air temperature was in the range from 12.3 to 23.5℃, and precipitation was 0.5 - 61.5 mm. The largest number of infections was recorded in May16 while in the months of June and April, they were, respectively, 12 and 4. In 2018, the average duration of precipitation periods was 5 hours. The number of periods lasting 1 hour was 15, and those lasting more than 1 hour - 17. The average duration of the infectious periods was 9 hours.

The experiment recorded 33 potential infectious periods in the second year. The shortest period was 1 hour, and the longest – 18 hours. Average temperatures ranged from 8.3 to 27.4℃, and precipitation from 0.2 to 69.2 mm. 12 infectious periods were recorded in June and April, and at least in May - 9. In 2019, the periods with precipitation had an average duration of 4 hours. The number of precipitations lasting 1 hour was 18, and those lasting more than 1 hour were 17. The average duration of long periods was 7 hours.

Potted trap plants placed in the orchard showed no symptoms for the entire two-year experiment. Under these circumstances, a definitive conclusion about the reliability of the model can hardly be made, but from a methodological point of view, it is appropriate to look for the reasons for the lack of symptoms.

One possible explanation is the low sensitivity of the method. Another possibility is that in the experiment in Plovdiv, the infectious background was not high enough. The size of the orchard in which the potted plants were exposed to potential infection in the first year was only 1.6 dka (0.16 ha). In the second year, infected leaves were collected and placed outside the orchard among which the potted trap plants were placed.

3. Control

3.1. Chemical treatments at different infection index thresholds

During the first half of the 2019 growing season, weather conditions were favorable for the development of CLS (Fig. 22). April was characterized by a large amount of precipitation (62.3 mm) and an average temperature of 12.5°C, which is close to the optimal for the development of the pathogen (Fig. 22a).

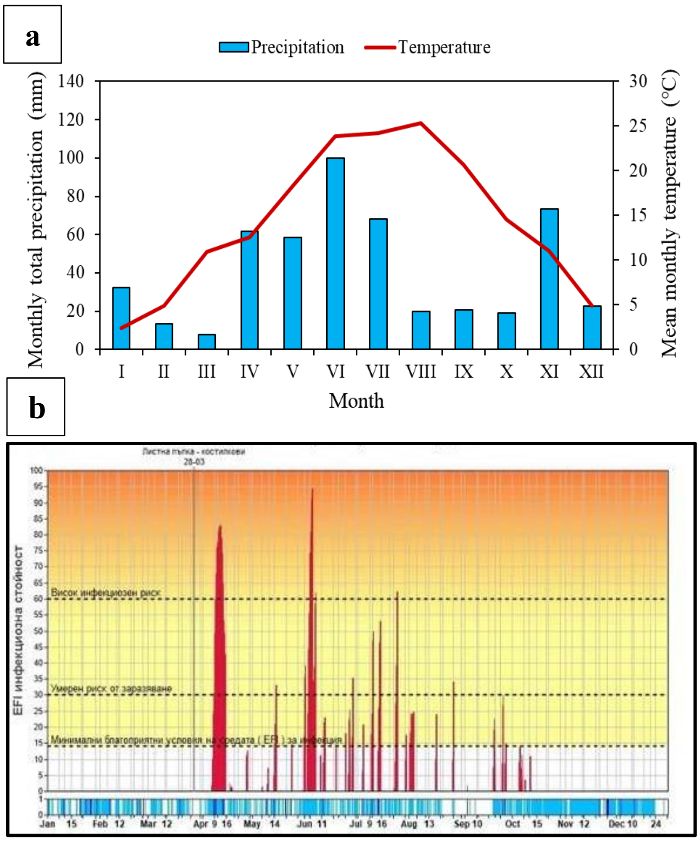

Figure 22. Meteorological conditions (a) and predicted infectious periods according to the Eisensmith and Jones model (b), Plovdiv, 2019. Source: RIMpro B. V., Netherlands.

The amount of precipitation in May was low – 18.7 mm, and for June it reached 138.6 mm. The average monthly temperatures for May (18.3℃) and June (23.9℃) were not limiting for the development of the disease. In the period from the beginning of April to the middle of June, two periods with a low index were recorded, one with an average and two with a high index (Fig. 22b). The first launches of single ripe ascospores were detected by spore trapping on 24.03. in the “green tip” stage (BBCH 09) in the cv. Van. In all treatments, the first treatment (11.04.) was during an infectious period with a high index (Table 22).

Table 22. Treatments for cv. Van variety, 2019

Date	Stage (BBCH)	Treatment [a]	Fungicide
11.04.	69	1, 2, 3	Luna Experience
25.04.	73	1, 2	Delan 700 VG
04.05.	75	1, 2	Delan 700 VG
13.05.	78	1, 2	Delan 700 VG
23.05.	81	1, 2, 3	Delan 700 VG
01.06.	85	1, 2, 3	Signum

[a] (1) Low index - EFI≥14; (2) Medium index - EFI≥28; (3) High index - EFI≥56

Spraying before infection with a contact product was not possible due to a change in the initial forecast indicating no risk. This necessitated the use of a fungicide with a curative effect – Luna Experience. The next four sprays were made with the contact product Delan 700 WG, and the last one is in view of the approaching harvest date with Signum.

First symptoms of the disease were detected in the untreated control on 3.5. during the stage "fruit growth" (BBCH 75). In the same month, due to low rainfall, only two infectious periods with an average index were recorded (14.05. and 24.05.), which did not significantly affect the development of the disease. Two CLS assessment were made on 26.05. and 05.06., respectively.

The analysis of the results includes not only their statistical significance but also the effect size (effect size) and the confidence interval. The effect size describes the importance of the observed effect and serves to determine its practical and theoretical value. Statistical significance does not always coincide with practical significance (Vasilev, 2014). The confidence intervals reported in some of the analyses serve to assess the uncertainty in determining the population mean.

The analysis of the CLS data produced a highly significant statistical result ($p_{KW} <$ 1%)for the effect size statistic, $\epsilon^2 = 0.18$, with a confidence interval of 0.18–1, indicating moderate explanatory power of the model. According to Dunn's test, there are statistically significant differences between the variants compared to the untreated control, and there are no such differences between the individual treatments (Table 23).

Table 23. Effect of treatments on CLS in 2019, cv. Van

Treatment	**CLS**
Untreated control	1.221 [a] a*
Low index (EFI≥14)	0.000 b
Medium index (EFI≥28)	0.000 b
High index (EFI≥56)	0.000 b

[a] Arithmetic mean of the reported severity (%).; * Treatments with different alphabetical designations have statistically significant differences in mean ranks (refers to the within-group characteristic) according to Dunn's test.

Studying the significance level of differences using the Varga and Delaney method confirms the results of the previous group of tests (Table 24).

Table 24. Significance of differences between treatments in 2019, cv. Van

Treatment I - Treatment II	**CLS**		
	VDA[a]	$L_{\mathbf{VDA}}$ [b]	$U_{\mathbf{VDA}}$ [in]
UNC – Low index	0.6489749	0.633928	0.663731
UNC – Medium index	0.6489749	0.633571	0.664074
UNC – High index	0.6489749	0.634224	0.663447
Low index – Medium index	0.4683068	0.448397	0.488317
Medium index – High index	0.5590098	0.539411	0.578425
Low index – High index	0.5272149	0.507773	0.546574

[*] UNC – Untreated control

[a] Varga-Delaney coefficient.; [b] Lower bound of the Varga-Delaney coefficient.;

[c] Upper limit of the Varga-Delaney coefficient.; * Intervals including 0.5 assume equal distribution of attack rate across treatments.

Compared to all other treatments, the untreated control shows the highest comparative probability of developing the disease. In the studied year, there were six treatments at the low and medium indexes and three at the high index. The results support the hypothesis that the treatment at a high index has a similar and no less effective result than that at a low index. The moderate explanatory power of the model in CLS shows that in addition to the treatment itself, there are other random factors that have an influence.

From a practical point of view, this means that the tested treatments under specific weather conditions and variety will give similar results if the experiment is repeated. Under other weather conditions, inoculum level, and variety, it is possible to obtain a different final result and effect for a given variant on the CLS. The study's data revealed that only the untreated control had infected leaves.

In addition to the leaves, symptoms of CLS were also found on fruit stalks. They were found during fruit ripening in the form of elongated spots measuring 1-2 mm, with a red to purple color and the presence of sporulation (summer macroconidia). To establish the effect of the treatments, one reading was performed on 8.6. The results of the analysis of variance of the data show a statistically significant effect $(P \approx 0)$. There is a statistically proven difference between the untreated control and the tested variants, and there is none between the individual variants (Table 25).

Table 25. Effect of treatments on CLS infection of fruit peduncles in 2019, cv. Van

Treatment	**Total number of fruit peduncles**	**Average (%)**		
Untreated control	1607	15.28 [a]	11.54 [b]	a[c]
Low index (EFI≥14)	622	1.25	0.45	b
Medium index (EFI≥28)	859	1.04	0.30	b
High index (EFI≥56)	773	0.03	0.10	b
$LSD_{0.05}$			3.078	

[a] Untransformed means (%)

[b] Data were transformed using arcisn[%]. Mean values in the table are presented in percentages after reverse transformation $\mathbf{sin^2}$(arcisn[%]).

[c] Treatments sharing at least one alphabetical designation do not have statistically significant differences in means.

From the presented results, it can be summarized that the treatments at high index have a comparable effect to those at low and medium index on both leaves and fruit stalks. In the specific year, the number of treatments at a high index resulted in half as many treatments compared to those at the other two lower indices.

In 2020, weather conditions during the first half of the growing season were favorable for the development of CLS. The first half of April was characterized by more frequent precipitation, while the second half was more rainless. Within the month, there were periods with suboptimal temperatures for the development of the pathogen (Fig. 23). The first ascospore discharge was detected by spore trapping on 24.03. in the “green tip” stage (BBCH 09).

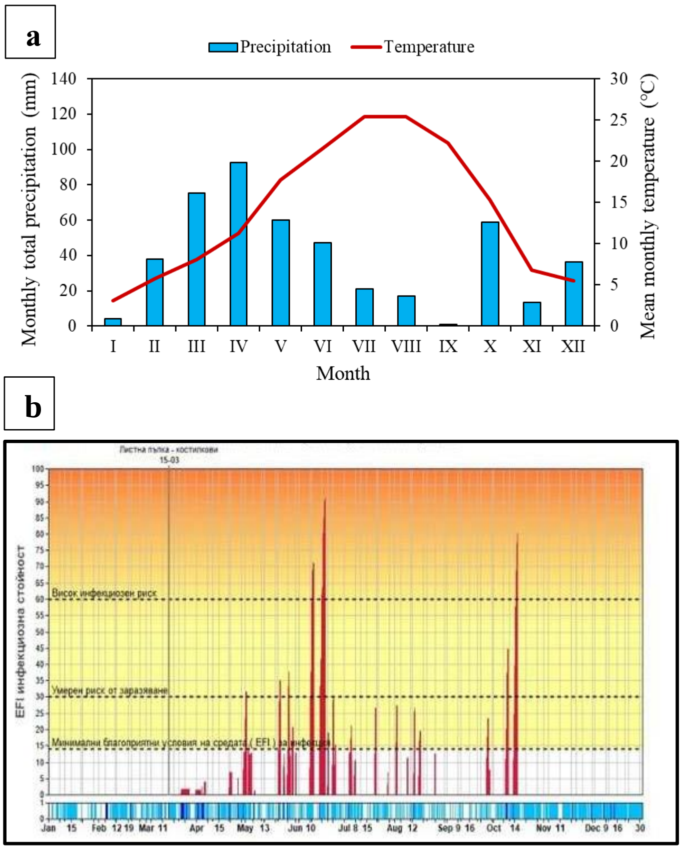

Figure 23. Meteorological conditions (a) and predicted infectious periods according to the Eisensmith and Jones model (b), Plovdiv, 2020. Source: RIMpro B. V., Netherlands.

The first treatment of the season was only for the treatment with a low index (30.04.), and the next two were for all treatments (Table 26). In view of the approaching harvest date, the last treatment was carried out with Signum.

Table 26. Treatments in 2020, cv. Van

Date	Stage (BBCH)	Treatment [a]	Fungicide
30.04.	73	1	Delan 700 VG
20.05.	77	1, 2	Delan 700 VG
31.05.	81	1, 2	Signum

[a] (1) Low index - EFI≥14; (2) Medium and high index - EFI≥28-56

The results for CLS are statistically highly significant $(p < 1\%)$, with a low effect size (Cohen, 1992) $(\epsilon^2 = 0.04)$with a confidence interval $[0.04, 1]$(Table 27).

Table 27. Effect of treatments on the severity of CLS in 2020, cv. Van

Treatment	CLS
Untreated control	3.61 [a] a [*]
Low index (EFI≥14)	0.07 b
Medium and high index (EFI≥28-56)	0.71 c

[*] Treatments with different alphabetical designations have statistically significant differences in mean ranks (refers to the within-group characteristic) in Dunn's test.
[a] Arithmetic mean of severity (%).

According to the VDA coefficient, the untreated control dominates stochastically both the “low index” treatment $(VDA = 0.69, [0{,}67, 0.71])$ and the “medium and high index” treatment $(VDA = 0.65,\ [0{,}63, 0.67])$ (Table 28). This shows that both treatments are better than the untreated control. Compared to the “medium and high index” treatment, the “low index” has more stable results $(VDA \approx 0.46,\ [0.435, 0.475])$.

Table 28. Significance of differences between treatments in 2020, cv. Van

Treatment I – Treatment II	**CLS**		
	VDA[a]	L_{VDA}[b]	U_{VDA}[c]
UNC – Low index	0.688365	0.67017	0.705997
UNC – Medium and high index	0.6476024	0.627691	0.667014
Low index – Medium and high index	0.4543462	0.434207	0.474636

[*] UNC – Untreated control

[**] Intervals including 0.5 assume equal distribution of severity across treatments.

[a] Varga-Delaney coefficient.

[b] Lower bound of the Varga-Delaney coefficient.

[c] Upper bound of the Varga-Delaney coefficient.

From the summarized data and the other analyses, it is evident that the difference between the two variants is small. The comparative analysis of the data from the two years shows that the treatments according to the Eisensmith and Jones predictive model before the infection period achieve several important goals. Firstly, contact products achieve better control of CLS and ensure there is no risk of resistance. On the other hand, it is possible to use curative products if proven necessary within a few hours after infection, e.g., if there is an unexpected change in weather conditions. An example of such a case was the beginning of the 2019 season when the first susceptible stage of the crop appeared. Last but not least, the number of treatments can be significantly reduced if they are applied according to the high infection risk index predicted by the model.

V. CONCLUSIONS

The results obtained from the experimental work carried out during the period 2018-2020 allow the following more important conclusions to be formulated:

1. The symptoms of CLS identified correspond to those described in the literature in our country and abroad; they were found on true and bract leaves, as well as on fruit stalks of cherry and sour cherry. The epiphytotic development of CLS in 2018-2019 led to premature defoliation and premature development of fruit buds and leaf buds in the period from late August to early September.

2. In terms of their morphological and cultural characteristics, the obtained isolates of *Cylindrosporium hiemalis* (Higgins) Trotter do not differ from each other and correspond to the descriptions in the literature. The same applies to the morphological features of AS, SPM, SMM, and microconidia taken from infected leaves during the study.

3. In individual years, the share of AS relative to the total number of primary spores caught during the season varies from 46 to 91% with an average value of 71%, and of SPM from 9 to 54% with an average value of the indicator of 29%.

4. In all years, the first AS and SPM were caught after the "green tip" stage in tree development. The first AS were recorded in the period from late March to mid-April, and SPM from late March to early May.

5. The annual dynamics of dispersal of AS and SPM differed between locations in a given year, as well as within a location in different years. The total number of days with SPM was smaller compared to that with AS present, which is probably related to their more limited share in the total mass of primary spores.

6. In all locations and years, 3-5 main peaks of AS and SPM dispersal per season were recorded, with the second type of spores being more pronounced. Droughts in April and May delayed the maturation of AS and SPM, which extended the dispersal period until the second half of June. By late May, they deplete the main spore reserves.

7. The main amount (85%) of AS and SPM was released from the fruiting bodies by the second half of May, and for the remainder, this event occurred during the first and third ten days of June.

8. In half of the cases, the threshold of 85% SPM dispersed in the air coincided with that of AS. In the remaining cases, a delay of 8 to 20 days was reported, with one exception, where the described event occurred 10 days earlier than AS.

9. The largest amount of both types of primary spores, taken separately, was reported in Plovdiv in 2019. The smallest numbers of AS and SPM were reported, respectively, in Brestnik in 2020 and in Botevgrad in 2018. The ratio between AS and SPM varied from 2:1 to 10:1, while in 2020 it was approximately 1:1.

10. The main part of AS and SPM was captured on days with precipitation, which proves the main importance of this factor for their dispersion. On days with no precipitation, cases in which only AS was present prevail; fewer are those with two types of spores present. In all likelihood, this is due to the dispersion mechanism, smaller size, and easy retention in the atmosphere in AS.

11. The largest number of events, with the first AS and SPM release, occur immediately after the start of precipitation. A smaller but significant share falls on launches that begin one or two hours after the specified moment. In the case of AS, the process may begin even 5 hours after the start of precipitation. The variation can be explained by the need for additional maturation in the presence of available moisture.

12. The dispersion of AS is less dependent on precipitation than that of SPM. AS releases are possible only in the presence of high leaf wetness and relative humidity higher than 90%, or only at high relative humidity (>90%). The amount of AS released under these conditions is approximately three times less than that provoked by precipitation.

13. The largest number of AS released was found in the interval from 1 to 13 hours after precipitation, and in the case of SPM, it is shorter, from 1 to 4 hours. During precipitation and after 13 hours, the amount of AS released is about 3–7 times lower. During precipitation and after the 4th hour, the amount of SPM released is 3-100 times lower.

14. The release of AS and SPM is possible at any time of the day. The presence of AS is greatest in the interval 01:00-07:00 h, while for SPM it is from 14:00 to 16:00 h.

15. In laboratory conditions, the duration of apothecia maturation at constant temperature and humidity has been established for 14 days (base temp. 0℃). The maturation of AS and SPM under equal conditions takes place for approximately the same period of time, respectively, 311 and 331 DD. The results of the experiment conducted in this way should be repeated due to the need for additional statistical proof.

16. The decomposition of fallen leaves had similar characteristics in different locations and years. The soil cover with leaves in the period from 30.11. to 30.3. remained still substantial (average 18.1%) , between 1.4. and 30.5. it decreased by an average of 35.6% and from 30.5. to 15.7. by 37.5%.

17. The development of CLS in terms of incidence and defoliation within a year were similar between individual locations within a season but different between years. Precipitation in June, as well as in July, is crucial for the development of the disease. There is no evidence that air temperature is a limiting factor.

18. Forecasting the development of the CLS using a two-way fixed effects model on panel data showed a strong relationship between average temperature and incidence and a less significant relationship for the other variables—precipitation and leaf wetness. The model shows potential, but from a statistical point of view, more information and reported indicators are needed, as it currently explains only 3% of the available data.

19. In field validation of the Eisensmith and Jones predictive model against primary infections, no symptoms were detected on the potted trap plants in both years of testing. A precise explanation cannot be given, but some of the assumptions are related to insufficient infectious background and small leaf volume.

20. The effect of chemical treatments at a high infectious index is comparable to that at low and medium, and the number of treatments in this case was reduced in 2019 by half—from 6 to 3, and in 2020 from 3 to 2.

21. Spraying at the end of the flowering stage (BBCH 69), when there is a risk of AS and SPM infection, is crucial for disease control for the rest of the season. All treated treatments at this stage in 2019 had significantly lower percentages of infected fruit stalks and leaves compared to the untreated control.

VI. DISSERTATION CONTRIBUTIONS

1. Original scientific contributions

1. For the first time in the world, a precise (hourly) study of the amount of spores in the air has been conducted using a high-end 7-day spore trap such as that of Burkard Manufacturing Co Ltd.
2. A differentiated measurement of AS and SPM in the air for the first time in the world has determined their ratio and dispersion dynamics during the day and growing season.
3. The release of SPM is possible at any time of the day and does not depend on light.
4. After a laboratory study, the required amount of degree days for the maturation of the fruiting bodies, apothecia, and spring acervuli was calculated.
5. For the first time in the world, a study has been conducted using an Eisensmith and Jones predictive model with built-in weather forecasting, which allows for a more flexible approach to decision-making and conducting preventive, rather than just curative, treatments.
6. For the first time in Europe, a study has been conducted to demonstrate infectious events under field conditions using potted trap plants.

2. Scientific contributions confirming other research

1. In Bulgarian conditions, the disease also develops symptoms on sweet and sour cherry fruit peduncles.
2. Discharge of AS in *B. jaapii* is possible at any time of the day.
3. In most years, the amount of AS is greater than SPM, but they may be equal.
4. It is possible to achieve a high level of protection from CLS through chemical treatment at a predicted medium and high infectious index from the Eisensmith and Jones model.

3. Practical contributions

1. Airborne AS and SPM epidemiological data can be used to create a mathematical model for primary infection risk assessment.

2. The largest number of events, with the first release of AS and SPM, occur immediately after the start of precipitation. A smaller but significant share falls on releases that begin one or two hours after the specified moment. Such results are an indication of the appropriateness of conducting chemical treatments with contact products against germinated but not yet infected spores during or shortly after precipitation. Such a practice is leading in the control of apple and pear scab.

3. Using the Eisensmith and Jones model in combination with a built-in weather forecast allows for optimal disease control after treatment when a high infectious index is reached, leading to a reduction in the total number of treatments per season. A similar strategy is applicable for management with biologically permitted or conventional products.

4. Data on the leaf litter decomposition may serve as a component of a predictive model for primary infection in CLS.

5. Chemical treatments during and after flowering, aimed at preventing infection on the stipules, are essential for controlling CLS throughout the season. During flowering, AS and SPM have been identified.

6. Confirmed infection on fruit stalks may become an additional infectious factor in orchards where produce is harvested mechanically and the stalks remain on the trees until spring.

7. The main stock of primary inoculum is released by the end of May, but a certain part of it is dispersed by the end of June. This proves the need for preventive spraying even during the harvest period and emphasizes the importance of all, including sanitary measures, beforehand that would reduce the infectious pressure.

Publications related to the dissertation

Marinov, M. (2022). Development of the cherry leaf spot epidemics in different regions of Bulgaria. *Agricultural Sciences/Agrarni* Nauki, *14* (32), 47-55.
DOI: 10.22620/agrisci.2022.32.008.

Participation in scientific and scientific-practical conferences

1. 1st International Symposium on Climate Change and Sustainable Agriculture, 14.11.-15.11.2019 – Plovdiv, Republic of Bulgaria.

2. VIII Congress on Plant Protection, 25.11.-29.11.2019 – Zlatibor, Republic of Serbia.

LIST OF ABBREVIATIONS AND SYMBOLS

Adj R - squared	Adjusted R-squared
AS	Ascospores
AU-Plovdiv	Agricultural University-Plovdiv
AWS	Automatic weather station
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical industry
CIPDM	Center for Integrated Plant Disease Management
CLS	Cherry leaf spot
DD	Degree Days
DSS	Decision support system
h	Hours
HTK	Hydrothermal coefficient
MA	Malt agar
mg mL –1	Milligram per milliliter
MMA	Modified malt agar
OA	Oat agar
PDA	Potato-dextrose agar
PPP	Plant protection products
SD	Standard deviation
SE	Standard error
SMM	Summer macroconidia
SPM	Spring macroconidia
TB	Temperature base