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Meteorological Conditions in a Temperate Climate for *Colletotrichum acutatum*, Strawberry Pathogen Distribution and Susceptibility of Different Cultivars to Anthracnose

Armina Morkeliūnė *, Neringa Rasiukevičiūtė and Alma Valiuškaitė

Laboratory of Plant Protection, Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai, LT-54333 Kaunas, Lithuania; neringa.rasiukeviciute@lammc.lt (N.R.); alma.valiuskaite@lammc.lt (A.V.)
* Correspondence: armina.morkeliune@lammc.lt

Abstract: Previously, *Colletotrichum* spp. has been considered a warmer climate pathogen as these meteorological conditions are most optimal for its development. However, climate change is fostering the spread of plant disease and complicating the ability to predict meteorological conditions for disease development. This study aims to determine meteorological conditions for anthracnose development, evaluate the susceptibility of different strawberry cultivars and detect the distribution of strawberry pathogens in temperate climate conditions. The experiment was carried out in the Institute of Horticulture Lithuanian Research Centre for Agriculture and Forestry (LAMMC) in Lithuania during the 2018–2019 strawberry growing season. To evaluate the contamination levels (fungal and bacterial pathogens) of strawberry plant parts, soil and susceptibility to *Colletotrichum acutatum*, samples were collected at four different locations in Lithuania from eleven cultivars. The results revealed that *Colletotrichum* spp. was not equally prevalent in the soil at all strawberry farms tested. The evaluation indicated that strawberry leaves and stems were similarly contaminated with pathogenic fungi. The most frequently isolated fungi from the leaves and stems were *Mycosphaerella* spp., *Alternaria* spp., *Fusarium* spp., *Colletotrichum* spp., *Phytophthora* spp., and *Botrytis* spp. Our study confirmed that the response of cultivar susceptibility to *C. acutatum* was unequal. The most suitable temperature for *C. acutatum* development was 25 °C. Monitoring of meteorological conditions, evaluation of inoculum source and appropriate cultivar selection could reduce or avoid yield losses caused by the *C. acutatum*.

Keywords: *Fragaria* × *ananassa*; iMETOS®; pathogenicity; plant parts; soil; temperature

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1. Introduction

Strawberries are one of the most popular dessert berries in the world due to their pleasant taste and aroma, as well as the fact that they contain many useful bioactive components [1]. Strawberry is the most important small fruit crop in Lithuania, produced on 560 hectares. With the growing consumption of fresh fruit and berries, it is becoming increasingly important not only to expand the range but also to extend the growing season of berries such as strawberries. As in other parts of the world, the strawberry business is expanding in Lithuania. Therefore, new varieties are being introduced from warmer climate zones. Strawberry growing is expanding not only outdoors, but also in greenhouses. Growing strawberries in greenhouses facilitate the spread of pathogens in tropical and subtropical climates.

One of the most common strawberry diseases is anthracnose. Strawberry anthracnose causes yield losses of up to 50% and up to 80% of plant death in nurseries [2]. The *Colletotrichum* species-genus comprises about 190 species that cause plant disease in various plant crops worldwide. Complexes of several *Colletotrichum* species cause strawberry anthracnose: *C. acutatum* J. H. Simmonds, brooks and *C. gloeosporioides* (Penz.) Penz. and Sacc. [3,4]. Anthracnose infects strawberry roots, crowns, petioles, leaves, runners, buds,

flowers and fruits. The *Colletotrichum* species complex has several development stages and infects different plant parts during their vegetation period [5–7]. *C. fragariae* mainly cause crown rot and lesions in vegetative tissue, and *C. acutatum* causes fruit rot [8]. The ability of these pathogens to attack different plant parts is due to the species complex involved in strawberry anthracnose [9–11].

Previously, strawberry anthracnose pathogen was considered to be a warmer climate pathogen, where temperatures ranging from 15 to 30 °C and optimal at 25 °C are needed for disease development. In comparison, the spread of *C. fragariae* is optimal between 26.7–32 °C. Temperature is a key factor for anthracnose development and occurrence [12–15]. *Colletotrichum* spp. mostly causes plant disease in tropical, subtropical and temperate regions around the world, as these meteorological conditions are most optimal for pathogen development [16]. Temperature influences the development of *Colletotrichum* spp. and appressoria formation [17]. Therefore, it is mostly not relevant in northern countries, except for warmer climate periods during berry harvesting and high rainfall [12,14,15]. However, *C. acutatum* causes berry infections at temperatures from 20 °C [13,18] and when there is a period of more than 12 h of leaf wetness [19]. The optimal temperature for anthracnose development is 12–27 °C and leaf wetness for more than 12 h [17,19]. Lithuania generally has temperate climatic conditions, hence the impact of *Colletotrichum* spp. should be minimal. According to our ongoing studies in Lithuania, the *Colletotrichum* spp. pathogen is not widespread, but due to climate change and its extremes, the occurrence of this disease is now more noticeable.

Due to climatic conditions, the battle against plant disease presents new challenges, and new strategies are required for plant protection. The prediction of meteorological conditions and the occurrence of plant diseases is a complicated science as it involves determining the relationship between meteorological conditions and plant disease risk. At the same time, host development may be influenced by climate change [20]. The growing demand for new solutions to optimise chemical pesticide usage and disease warning systems will not only help to monitor disease risk but also to predict disease epidemics. Meteorological parameters used to predict crop disease occurrence include air temperature, leaf wetness, precipitation and relative humidity. Disease forecasting models determine the critical timing of fungicide application when the conditions are most favourable for disease development. This also avoids unnecessary spraying and applications are made when the conditions are favourable for disease. Disease forecasting models are based on the impact of temperature and leaf wetness duration [7,19,21,22]. There are various disease forecasting models for specific plant diseases. This study summarises the meteorological conditions for *C. acutatum* to determine the risk of strawberry anthracnose infection [8]. The anthracnose fruit rot (AFR) model shows that disease control based on an advisory system could significantly reduce the number of sprayings [23]. Similarly, the StAS web-based forecasting system has the potential to reduce spraying times compared with fixed-interval applications [13,24]. However, all forecasting models are based on counting the favourable periods for disease development. In Lithuania, IMETOS® meteorological stations with forecasting models were used for a while [21,22,25,26]. The disease forecasting models are not only beneficial for the farmer (more precise application) but also for the environment (fewer pesticides, accurate applications).

The sources of a fungal infection include previous crops, contaminated soil, irrigation water and farmworkers. Soilborne pathogens can be species-specific, while resistance to anthracnose differs among strawberry varieties. Soilborne pathogens cause up to 20–30% yield losses in strawberry crops and can survive in the soil for several years [27–29]. Strawberry disease control generally relies upon several fixed-interval (7–10 days) applications of fungicides from the time of leaf emergence until harvest, thus requiring significant amounts of fungicides [7,22,30], which are costly and ideally should be applied when the risk of infection is high. The growing resistance to pesticide use and their adverse environmental effects are leading to new, environmentally safer disease control strategies [7,31,32]. The selection of resistant cultivars (cv.) to anthracnose reduces the inoculum levels; additionally,

meteorological conditions' monitoring could assist in controlling the spread of *C. acutatum*. This study aims to determine the meteorological conditions for anthracnose development, evaluate the susceptibility of different strawberry cultivars and detect the distribution of strawberry pathogens in temperate climate conditions.

2. Materials and Methods

2.1. Colletotrichum sp. Isolate

The isolate of *Colletotrichum* sp. used in this study were collected from the infected strawberry cultivar 'Deluxe' fruit grown in the experimental strawberry field of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry (LAMMC). The *Colletotrichum* sp. mycelium was maintained by sub-culturing on potato-dextrose agar (PDA) at 25 °C for 7 days. This step repeated twice to purify culture, then single-spore isolates were extracted. Single spore *Colletotrichum* sp. isolates were stored on PDA at 4 °C at the Laboratory of Plant Protection isolate collection. The single spore isolate Nr. Fo5 was initially identified by its morphological attributes.

Colletotrichum sp. DNA was extracted as described by Rasiukevičiūtė et al. [33]. The DNA was extracted using a genomic DNA purification kit (Thermo Fisher Scientific Baltics, Vilnius, Lithuania). DNA was dissolved in 100 µL of 1 × TE buffer and visualised in 1.5% agarose gels with Midori Green Direct (Nippon Genetics Europe). DNA concentration was measured by NanoDrop 1000 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). C. acutatum isolates were identified using species-specific primers ITS4 (TCCTCCGCTTATTGATATGC-3) and CaInt2 (GGGGAAGCCTCTCGCGG) primers [34]. Polymerase chain reaction was performed in a total volume of 25 µL containing 1 µL of DNA, 5 µL 10 × Tag buffer, 0.5 µL 10 mM dNTP Mix, 0.5 µL of each primer (CaInt2 and ITS4), 1.5 µL 25 mM $MgCl_2$, 0.2 µL 5 U μL^{-1} Taq DNA polymerase (Thermo Fisher Scientific), and 15.8 µL DNase/RNase-free water. The PCR was performed in UNO96 thermal cycler (VWR International GmbH), for 35 cycles consisting of 1 min at 95 °C, 30 s at 54 °C, and 1 min at 72 °C. Amplification products were separated in 1.5% agarose gels, stained with Midori Green Direct (Nippon Genetics Europe).

2.2. iMETOS® Meteorological Conditions

This research was conducted at the LAMMC in 2018 and 2019. We evaluated data from iMETOS® (Pessl Instruments, Weiz, Austria) meteorological stations at four different locations in Lithuania over four months (May, June, July and August) in 2018 and 2019 to determine the favourable conditions for *C. acutatum* spread (Table 1). Farms 1 and 2 were evaluated in 2019, and Farms 3 and 4 in 2018 and 2019. The studied plant material was from 2–3 year-old plants.

Table 1. The iMETOS® meteorological station locations in Lithuania and strawberry cultivars.

District	Region	Farm Name	Grown Cultivars
Radviliškis distr., GPS 55.892954, 23.870937	North	Farm 1	Florence, Asia, Rumba, Vibrant, Polka, Malvina
Šiauliai distr., GPS 55.740328, 23.519661	North	Farm 2	Flair, Asia, Malvina
Šiauliai distr., GPS 55.9712991, 22.9108421	North	Farm 3	Senga Sengana, Rumba, Polka, Malvina
Kaunas distr., GPS 55.084567, 23.806315	Central	Farm 4	Asia, Rumba, Sonata, Malvina, Elkat, Deluxe

The production of strawberry fruit in Lithuania occurs from mid-April (June-bearing) until late September (everbearing). iMETOS® meteorological stations are equipped with various sensors (air and soil temperature, precipitation, leaf wetness, relative humidity). The *C. acutatum* infection risk periods were determined according to the meteorological

conditions. Favourable days for disease development were counted if the air temperature was between 12–27 °C and the leaf wetness period lasted more than 720 min (12 h) [8,35].

2.3. Strawberry Plant Contamination

Asymptomatic strawberry samples were randomly collected in June–July 2018 and 2019 (Table 1). Ten different strawberry cv. were evaluated in a strawberry plant contamination study. The samples of leaves and stems were cut into 1 cm fragments, surface-sterilised for 3 min in 70% ethanol and rinsed five times with sterile distilled water (dH_2O). Leaves and stems were analysed separately. The plant fragments were placed on potato dextrose agar (PDA, for fungi) and plate count agar (PCA, for bacteria) and incubated at 25 °C. Contaminant colony units were converted to percentages (%) and identified according to the morphological traits typical of the colonies' descriptions under a microscope after 3, 5 and 7 days post-inoculation (DPI). One replication consisted of 50 leaves or stems per cultivar with four replicates per treatment.

2.4. Soil Contamination

Soil samples were collected randomly in four replicates per cultivar (Table 1). Ten iferent strawberry cv. were evaluated in the soil contamination study. Soil suspension dilutions were prepared to evaluate the number of colony-forming units (CFU) of bacteria and fungi in the soil by the plate count method. The soil (10 g) was diluted with 90 mL dH_2O to concentrations up to 10^{-6}. Each sample was replicated twice. The dilutions of 1 mL series were plated onto PDA (for fungi) and PCA (for bacteria) and incubated at 25 °C. The total number of bacteria and fungi was evaluated after 3, 5 and 7 DPI. The total CFU was converted to a log value (CFU/g^{-1}) [36]. All experiments (plant isolations and soil dilution studies) were conducted twice during the strawberry production season on 14 June 2018 and 18 July 2018, and 20 June 2019 and 25 July 2019. Data from soil experiment are provided as an average of four sampling dates.

2.5. Effect of Temperature of Growth C. acutatum

Single spore isolate of *C. acutatum* was maintained by sub-culturing on PDA at 25 ± 2 °C for seven days. Six mm diameter mycelial plugs were cut and placed in the centre of new Petri dishes containing PDA. The plates were incubated at 5, 10, 15, 20, 22 and 25 °C in darkness. Growth rates were determined by measuring the fungal colony diameter (cm) at 2, 4 and 7 days post-inoculation (DPI). Four replicates were used in each treatment, and the experiment was repeated twice.

2.6. Strawberry Cultivars' Susceptibility to C. acutatum

The susceptibility to *C. acutatum* was determined on the detached strawberry leaves. Leaves were obtained from Farm 4 in 2019. The six selected strawberry cvs. were: 'Malvina', 'Asia', 'Deluxe', 'Sonata', 'Elkat' and 'Rumba'. Visually healthy strawberry leaves (16 leaves per replicate, four replicates in one cultivar), composed of three leaflets on a petiole, and without any visible disease symptoms were sterilised in 70% ethanol solution for 3 min, rinsed five times with sterile distilled water (SDW) and dried for 5 min on sterile filter paper. The upper surface of the leaf was inoculated with 5 mm mycelial plugs (mycelial side down) in the centre of each multiple leaves. The experiment was repeated four times, with four replicates. The Petri plates were incubated at 25 °C in darkness for 15 DPI. The disease severity index of each inoculated leaf was assessed up until 15 DPI using the following severity scale: (1) 0%—no visible infection; (2) 5%; (3) 10%; (4) 20%; and (5) 50% or more infected area [37].

2.7. Statistical Analysis

The data were analysed using an analysis of variance ANOVA test with SAS Enterprise Guide, version 7.1 (SAS Institute Inc., Cary, NC, USA). The standard error in the figures is marked as an error bar estimated for growth rates of isolates. Duncan's multiple range

test ($p < 0.05$) was used to determine differences among treatments. Two-way Anova was used to determine differences in *C. acutatum* mycelium growth based on two factors: day and temperature.

3. Results

3.1. iMETOS® Meteorological Station Conditions

A comparison of meteorological conditions was performed for four farm locations in 2018 and 2019 during May, June, July and August. Meteorological conditions for *C. acutatum* infection development are provided in Figure 1. In 2018 and 2019 in Lithuania, strawberry vegetation seasons had quite similar climatic conditions, a relatively warm temperature with low precipitation and low air humidity. However, evaluating the data from iMETOS® meteorological stations in 2018 and 2019 indicated different conditions for strawberry *C. acutatum*.

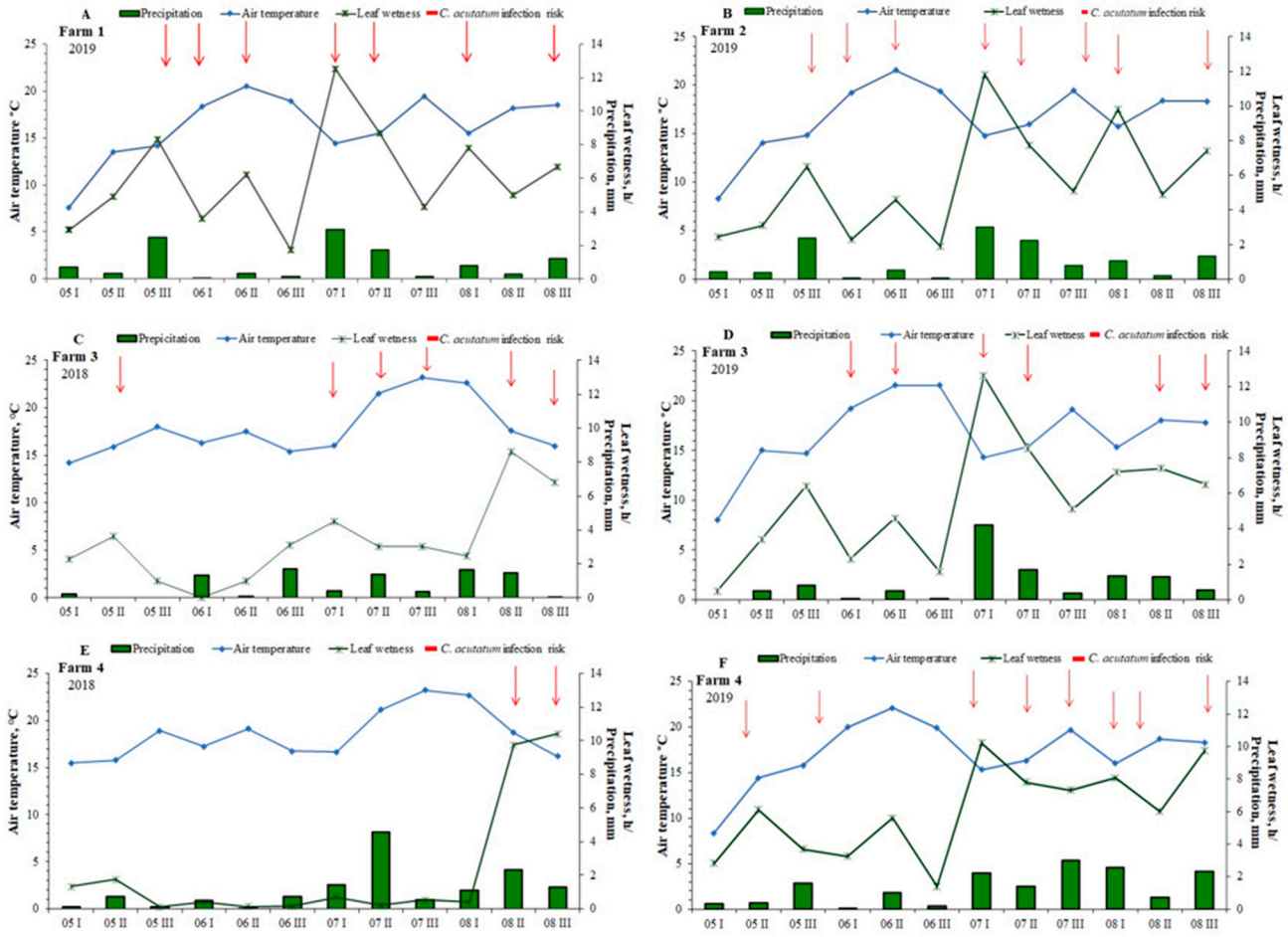

Figure 1. The meteorological conditions according to iMETOS® meteorological stations ((**A**) Farm 1 in 2019; (**B**) Farm 2 in 2019; (**C**) Farm 3 in 2018; (**D**) Farm 3 in 2019; (**E**) Farm 4 in 2018; (**F**) Farm 4 in 2019). Data are presented as an average.

The iMETOS® meteorological conditions for strawberry anthracnose of 2018 are provided in Figure 1. In 2018, the risks of infection in Farm 3 (Figure 1C) lasted for ten days: one day in May, four days in July and 5 days in August (Table 2). The air temperature, precipitation and leaf wetness parameters did not create favourable conditions for the spread of anthracnose for three months in Farm 4 (Figure 1E). Analysis of the iMETOS® data records showed that conditions for strawberry anthracnose in 2018 were favourable in August; 5–7 days were suitable for the spread of infection (Farms 4 and 3) (Table 2). The following factors determined that the risk periods in 2018 for infection: air temperature between 12.0–25.1 °C and a leaf wetness period lasting more than 720 min.

Table 2. Conditions for strawberry C. acutatum development, according to iMETOS® meteorological station data in 2018.

Infection Conditions	Farm 3				Farm 4			
	May	June	July	August	May	June	July	August
Air temperature min-max, °C	10.6–21.7	9.4–20.9	11.5–24.8	13.4–25.1	11.5–22.5	11.6–22.0	11.9–25.3	13.2–24.9
Leaf wetness period, min	0–925	0–430	0–990	0–1180	0–685	0–240	0–330	0–1440
Total favourable days	1	0	4	5	0	0	0	7
Risk days (Days of the month)	15		2, 3, 13, 30.	11, 12, 14, 25, 26.				11, 12, 15, 25, 26, 27, 29.

The analysis of iMETOS® meteorological station data showed that, in 2019, favourable conditions for the spread of *C. acutatum* infection occurred during strawberry flowering at the end of May (for 3–4 days) in Farms 1, 2 and 3. (Table 3). The meteorological station data for 2018 were different compared to 2019. The highest air temperature was in June, but the amount of precipitation and leaf wetness was low. Therefore, the risk of infection lasted 2–3 days but did not occur in Farm 4. However, sufficient precipitation, the temperature and leaf wetness conditions in July created favourable conditions for the spread of *C. acutatum* (favourable for 8–11 days). The risk of infection lasted 4–7 days in August (Table 3). The following factors determined the risk periods for infection: air temperature between 12–25.8 °C and a leaf wetness period lasting more than 720 min.

Table 3. Conditions for strawberry *C. acutatum* development, according to iMETOS® meteorological station data in 2019.

Infection Conditions		Air Temperature Min-Max, °C	Leaf Wetness Period, Min	Total Favourable Days	Risk Days (Days of the Month)
Farm 1	May	3.8–20.1	0–1375	4	23,27,28,29.
	June	15.1–24.7	0–1015	3	1,14,17.
	July	12.1–22.9	0–1440	8	4,5,7,8,9,10,16,17.
	August	13.6–21.4	0–990	6	8,9,10,21,22,30.
Farm 2	May	4.3–30.8	0–1425	3	23,27,28.
	June	15.1–25.8	0–1000	2	1,17.
	July	12.5–23.1	0–1430	8	5,7,8,9,10,16,17,22.
	August	14.2–22.1	0–995	5	8,9,21,22,30.
Farm 3	May	4.3–21.6	0–1345	0	
	June	15.1–25.8	0–1000	2	1,17.
	July	12.5–23.1	0–1430	8	5,7,8,9,10,11,16,17
	August	14.2–22.1	0–920	5	9,10,14,15,21.
Farm 4	May	5.2–22.1	0–1055	3	11,29,31.
	June	16.4–26.3	0–680	0	
	July	12.7–22.8	0–1375	11	3,7,8,9,10,11,16,17,18, 22,27.
	August	14–22.1	0–1025	7	2,9,10,11, 21,22,30.

Meteorological data for 2019 showed that the most favourable conditions for the spread of anthracnose occurred at the end of May and in the first ten days of July. However, the prevailing temperature was below the optimum. Meteorological data showed that the favourable conditions for strawberry anthracnose spread varied in different years. Sufficient precipitation and long periods of leaf wetness provided suitable conditions for the spread of *C. acutatum* infection. Therefore, it can be stated that, in a colder climate, the temperature favourable for the spread of anthracnose is between 15.0–22.0 °C. According to the meteorological data obtained in Farms 1 and 2, the conditions for the spread of

strawberry anthracnose differ very slightly in 2019. More favourable conditions for the spread of *C. acutatum* infection occurred at the beginning of strawberry vegetation.

3.2. Strawberry Contamination

A study of the contamination of different parts of strawberry plants with pathogenic fungi showed that infestation of strawberry leaves and stems was similar (Figure 2). The results from Farm 1 showed that the highest contamination with pathogenic fungi was observed in cv. 'Malvina' (75.3% and 65.8%, stems and leaves, respectively), with the contamination being evenly distributed on leaves and stems. The lowest contamination was observed in cv. 'Vibrant' plant parts. Contamination of plant parts was similar in cultivars in Farm 2. Cv. 'Rumba' grown in Farm 3 had a high infestation in its stems and leaves (69% and 47.2% respectively).

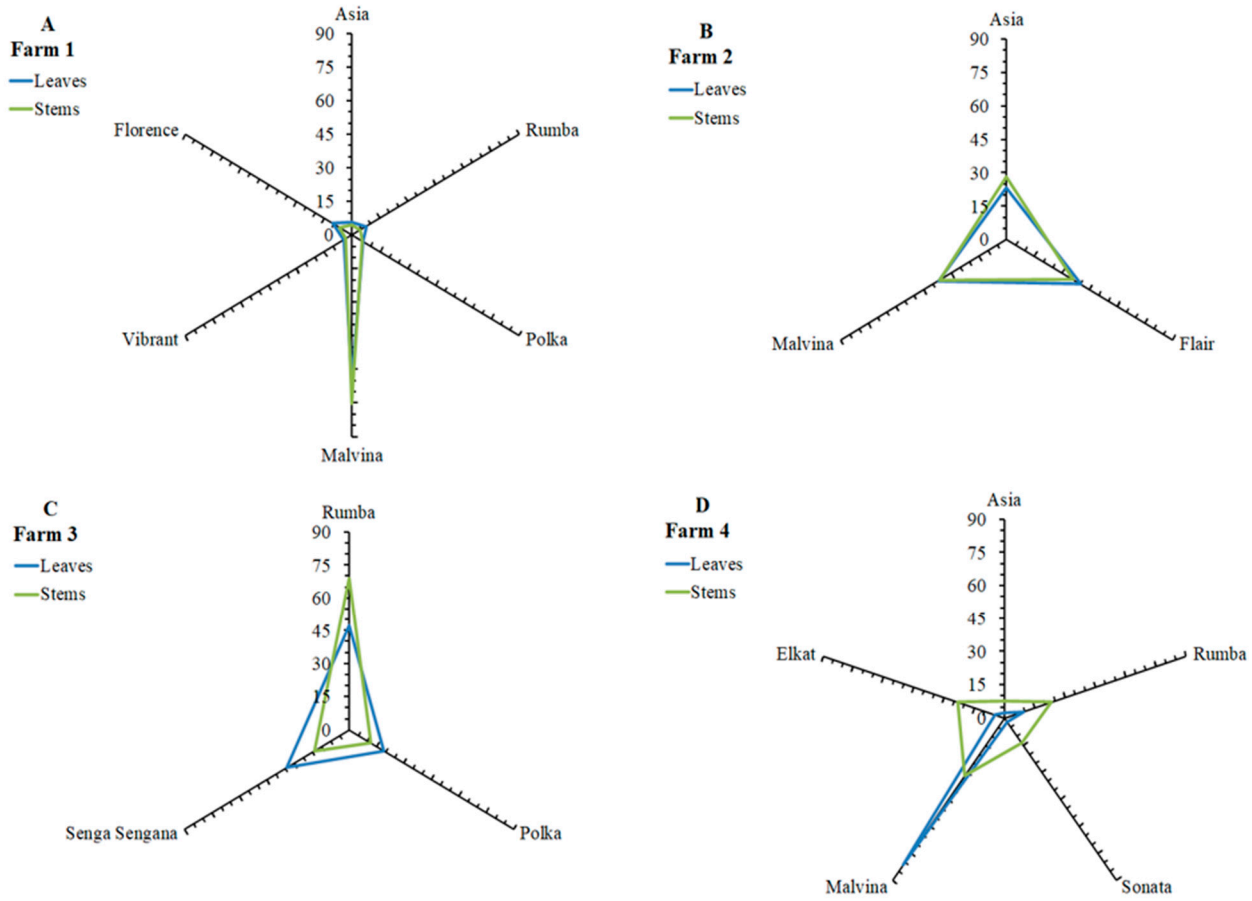

Figure 2. Fungal contamination of various strawberry cultivars leaves and stems (%). (**A**) Farm 1 cv. 'Asia', 'Rumba', 'Polka', 'Malvina', 'Vibrant', 'Florence'; (**B**) Farm 2 cv. 'Asia', 'Malvina', 'Flair'; (**C**) Farm 3 cv. 'Rumba', 'Polka', 'Senga Sengana'; (**D**) Farm 4 cv. 'Asia', 'Rumba', 'Sonata', 'Malvina', 'Elkat'. Results are presented as means (n = 4).

The results differed between leaves and the stem in different strawberry cv. The contamination was highest when comparing the obtained results in the same cvs., for example, 'Rumba' and 'Malvina', grown in different farms (Figure 2).

Evaluation of strawberry plant parts indicated that strawberry leaves and stems were similarly contaminated with pathogenic fungi. Differences between strawberry cultivars were observed. The highest contamination with pathogenic fungi was observed in 'Malvina' stems and leaves in two strawberry farms. Contamination of 'Rumba' plant parts with pathogenic fungi was lower than 'Malvina' in Farms 1 and 4. However, results obtained in Farm 3 showed contamination of the stems and leaves with pathogenic fungi in cv. 'Rumba' was relatively high.

Our data indicate (Table 4) that the isolated fungi were *Mycosphaerella* spp., *Alternaria* spp., *Fusarium* spp., *Colletotrichum* spp., *Mucor* spp., *Penicillium* spp., *Phytophthora* spp., *Botrytis* spp. and that *Trichoderma* spp. was the most frequently isolated fungus from the leaf and stem samples of strawberry plants collected from cv. 'Rumba', 'Asia', 'Florence', 'Malvina' and 'Senga Sengana'. The *Colletotrichum* spp. pathogen was most frequently found in cv. 'Malvina' compared with other tested cultivars. Identification was carried out according to the cultural and morphological characteristics.

Table 4. Frequency (%) of isolated fungi from the leaves and stems of strawberry plants, 2018–2019.

Isolated Fungi/Cultivar	Frequency of Isolated Fungi (%)									
	No.	%	No.	%	No.	%	No.	%	No.	%
	Rumba		Asia		Florence		Malvina		Senga Sengana	
Mycosphaerella spp.	0	0	0	0	0	0	21	13.9	0	0
Alternaria spp.	4	4.3	41	29.7	7	7.4	10	6.6	22	41.5
Unknown	10	10.9	27	19.6	11	11.7	27	17.9	6	11.3
Fusarium spp.	46	50	36	26.1	9	9.6	40	26.5	14	26.4
Colletotrichum spp.	2	2.2	2	1.5	3	3.2	5	3.3	1	1.9
Mucor spp.	1	1.1	5	3.6	0	0	14	9.3	3	5.7
Penicillium spp.	15	16.3	12	8.7	51	54.3	10	6.6	4	7.5
Phytophthora spp.	12	13	8	5.8	4	4.2	8	5.3	1	1.9
Botrytis spp.	0	0	2	1.4	9	9.6	3	2	0	0
Trichoderma spp.	2	2.2	5	3.6	0	0	13	8.6	2	3.8
Total	92	100	138	100	94	100	151	100	53	100

No. = Number of isolated fungi; % = Percentage count of isolated fungi.

3.3. Soil Contamination

Soil samples from leading strawberry farms were collected to determine the prevalence of pathogens in the soil, in which the different strawberries cultivars were grown. During our research, 571 bacteria and only 99 fungal samples were isolated from the soil. The species of fungi detected were: *Colletotrichum* spp., *Fusarium* spp., *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Botrytis* spp., *Mucor* spp. and others. The bacteria CFU was higher than fungi in all strawberry cultivars. The total amount of colony-forming units detected in the soil is presented in Table 5.

Table 5. The total amount of colony-forming units (CFU g^{-1}) in strawberry soil from different farms. Means followed by the same letter did not differ significantly ($p < 0.05$).

Farm	Total Amount, CFU/g^{-1}		
	Cultivar	Bacteria	Fungi
Farm 1	´Florence´	3.98 ± 0.69 cd	3.60 ± 0.40 bcd
	´Asia´	4.14 ± 0.48 d	3.44 ± 0.82 bcd
	´Rumba´	3.92 ± 1.17 bcd	3.24 ± 0.29 ab
	´Vibrant´	4.13 ± 1.02 d	3.30 ± 0.63 ab
Farm 2	´Flair´	3.48 ± 0.27 abc	3.00 ± 0.63 a
	´Malvina´	3.48 ± 0.62 abc	3.10 ± 0.64 ab
Farm 3	´Rumba´	4.05 ± 0.50 d	3.18 ± 0.29 ab
	´Polka´	3.18 ± 0.58 ab	2.70 ± 0.41 a
	´Asia´	3.18 ± 0.64 ab	3.00 ± 0.71 a
Farm 4	´Elkat´	4.17 ± 0.87 d	3.72 ± 0.91 d
	´Malvina´	2.87 ± 0.85 a	3.60 ± 0.63 bcd
	´Sonata´	4.49 ± 0.41 f	2.70 ± 0.27 a
	´Deluxe´	4.49 ± 0.65 f	3.10 ± 0.25 ab

Results are presented as means $n = 4 \pm$ SE.

The lowest CFU of bacteria was determined in a soil sample taken from Farm 4 (2.87×10^3 CFU/g^{-1}), where various strawberry cv. were grown, but this was particularly low for the 'Malvina' cv. However, the CFU of fungal pathogens was one of the highest (3.60×10^3 CFU/g^{-1}). An increase in bacteria CFU was found in 'Sonata' (4.49×10^3 CFU/g^{-1}) and 'Deluxe' (4.49×10^3 CFU/g^{-1}) from Farm 4 soil samples. Farm 4 'Sonata' and Farm 3 'Polka´ had the lowest CFU of fungi (CFU 2.70×10^3 CFU/g^{-1}). Among all evaluated soil samples from different farms, the highest CFU of fungi were found in Farm 4 'Elkat' (3.72×10^3 CFU/g^{-1}). The soil contamination of bacteria ranged from 3.92 to 4.14×10^3 CFU/g$^{-1,}$ and fungal pathogens ranged from 3.24 to 3.60×10^3 CFU/g^{-1} among different strawberry cv. in Farm 1. In this study, we found that 'Rumba' had similar contaminations of bacterial and fungal pathogens in different growing locations. However, in Farm 1, 'Asia' had significantly higher contamination of bacterial and fungal pathogens than those cultivated in Farm 4. The 'Polka' in Farm 3 was the least infected with soil pathogens compared with other cultivars. The lowest CFU of soil pathogens was detected in Farm 3 compared with Farms 1, 2 and 4. Soil contamination indicates that there were higher concentrations of pathogens. *Colletotrichum* spp. was observed in only three soil samples, that is, one sample from Farm 4 and 2 samples from Farm 1. From our results, it can be concluded that the anthracnose that causes *Colletotrichum* spp. was not equally prevalent in the soil of all strawberry farms. Our data show that bacterial and fungal pathogens distribution varies in different cultivars and locations. However, as some cultivars are more susceptible to some pathogens, their distribution was higher.

3.4. Effect of Temperature on Colletotrichum acutatum Growth

Temperature is one of the essential factors influencing *Colletotrichum* spp. growth. The isolates of *C. acutatum* showed significant differences in fungal growth under different temperature conditions. The highest colony growth rate was observed at 25 °C, and the lowest at 5 °C. The optimal temperature range was 20–25 °C, and the maximum growth rate was from 4.41 to 6.01 cm, respectively. Significant differences were observed between temperatures of 20–25 °C at 7 DPI. *C. acutatum* mycelium growth differs by only 0.03 cm at 5 °C after 4 and 7 DPI. The mycelium of *C. acutatum* was able to grow at 5 and 10 °C, but growth was slower compared with other temperatures. Furthermore, no visible colonies were observed at 2 DPI at 5 and 10 °C. Our data revealed that at 10 °C (1.20 cm), *C. acutatum* mycelium grew faster compared with 5 °C (0.6 cm) at 4 DPI. The mycelium diameter was 0.81, 1.61 and 2.73 cm after 2, 4 and 7 DPI at 15 °C, respectively. No significant differences were observed at 2 and 4 DPI. However, the highest growth was observed at 25 °C after 2, 4 and 7 DPI (1.76, 3.35 and 6.01 cm, respectively).

The results of our experiment indicate significant differences in fungal growth under different temperature conditions. In vitro temperature tests revealed that the optimal temperature for *C. acutatum* development was between 20–25 °C. However, the most suitable temperature for *C. acutatum* development was 25 °C (Table 6).

Table 6. Effect of temperature on *C. acutatum* mycelium growth after 2, 4 and 7 DPI. DPI–days post-inoculation.

	***C. acutatum* Mycelium Growth, cm (Factor A)**			
Temperatures, °C (Factor B)	**2DPI**	**4DPI**	**7DPI**	**Average B (p = 0.064)**
5	0.00 ± 0.00	0.80 ± 0.00	0.83 ± 0.02	0.54 **
10	0.00 ± 0.00	1.20 ± 0.02	2.44 ± 0.04	1.21
15	0.81 ± 0.02	1.61 ± 0.03	2.73 ± 0.04	1.71
20	1.25 ± 0.05	2.68 ± 0.05	4.41 ± 0.11	2.78 **
22	1.24 ± 0.11	2.90 ± 0.06	4.87 ± 0.10	3.00
25	1.76 ± 0.08	3.35 ± 0.05	6.01 ± 0.13	3.71 **
Average A (p = 0.054)	0.84	2.09	3.55 **	2.16

** statistically significant differences at $p < 0.05$. DPI—Days post-inoculation. Results are presented as means $n = 4 \pm$ SE.

3.5. Susceptibility of Strawberries to C. acutatum

The detached strawberry leaf assay was developed to determine the susceptibility of strawberry cv. to *C. acutatum.* The results revealed that *C. acutatum* isolate from strawberry was able to cause anthracnose in tested cv. and were pathogenic. Clear *C. acutatum* necrotic (black) areas developed around mycelial plugs on inoculated leaves, however, no necrotic lesion was observed on control leaves. The 'Elkat' and 'Deluxe' cv. showed the highest susceptibility (4.64 and 4.33, respectively). The cultivars 'Malvina' and 'Sonata' had a lower disease severity index (3.77 and 2.89, respectively) and were not significantly different. The 'Rumba' (1.50) and 'Asia' (1.83) cv. had a significantly lower disease severity index (Figure 3).

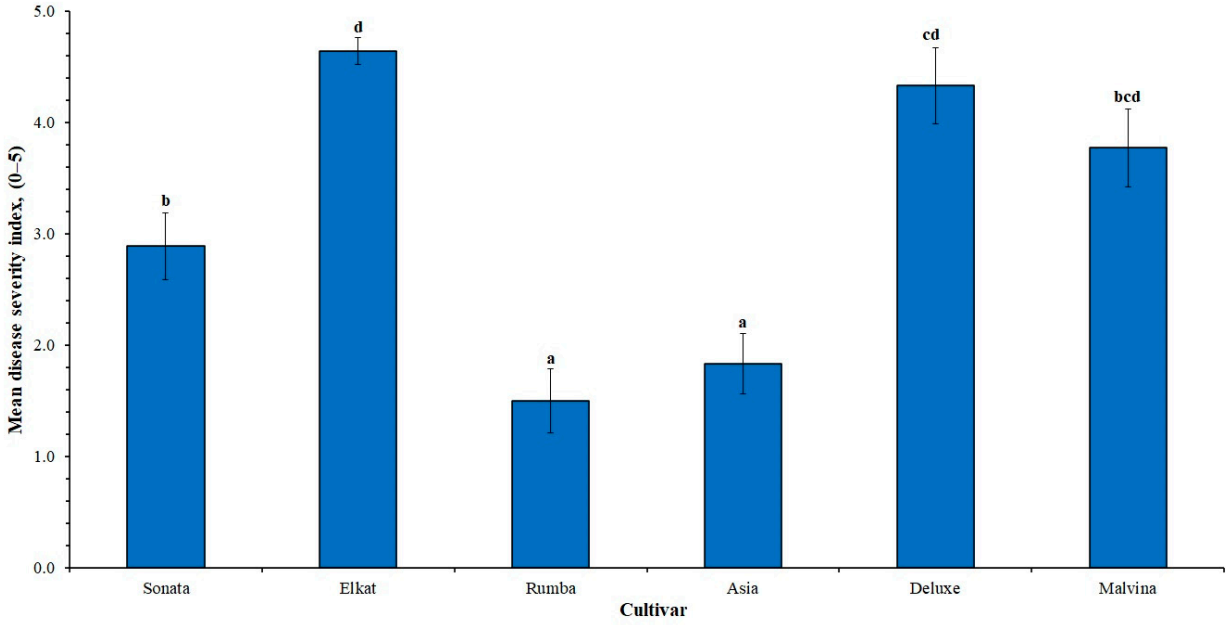

Figure 3. The susceptibility of different strawberry cultivars inoculated by *C. acutatum.* Means followed by the same letter did not differ significantly ($p < 0.05$).

4. Discussion

Climate change is promoting the spread of plant disease, and the spread of the anthracnose pathogen is noticeable. It is becoming complicated to predict meteorological conditions as well as disease development. The variation between rainfall, temperature and leaf wetness affects plant diseases [20]. The temperature and relative humidity are potentially significant factors affecting the development of *Colletotrichum* species complexes. We evaluated the meteorological conditions in a temperate climate for *C. acutatum* development. Our study showed that meteorological conditions have become more favourable for *C. acutatum* [7,8,19,21,22,35]. Disease forecasting models help predict disease risks and reduce fungicide application by 50%. The anthracnose advisory system based on the AFR model showed that it could significantly reduce the number of sprayings [23,38]. Rasiukevičiūtė et al. [22] evaluated the iMETOS® strawberry *Botrytis cinerea* forecasting model. Their results were very promising, as the pesticide applications were reduced based on the forecasting model. Our results show that meteorological conditions vary in different areas and years. We found that there were an optimal 12 days in 2018 and 26 days in 2019 for the development and spread of anthracnose in strawberry plants. The anthracnose infection risk in Farm 3 in 2018 lasted for 10 days in May-August and for seven days in Farm 4, only in August. Meanwhile, in 2019, in Farms 1 and 4 it lasted 21 days in May-August, in Farm 2 for 18 days and 15 days in Farm 3.

Meteorological conditions influence the conditions for disease development. However, there is always a source of infection. The primary source of infection is contaminated soil

and previous crops. Soilborne pathogens reduce strawberry yield by up to 20–30% [27–29]. We conducted a series of experiments to evaluate soil and the contamination of strawberry plant parts by pathogens. Soil contamination shows that there are higher concentrations of pathogens. In our research, 571 bacteria and only 99 fungal samples were isolated from the soil. The number of bacteria in the soil was higher than that of fungal pathogens. However, *Colletotrichum* spp. was not equally prevalent in the soil at all tested farms.

Strawberry plant part evaluation indicated that the contamination of strawberry leaves and stems was similar. The highest level of contamination by pathogenic fungi was observed in cv. 'Malvina' and 'Rumba' stems and leaves. According to the results obtained, it can be stated that 'Asia' leaves and stems were least infected with pathogenic fungi. Wagner et al. [6] indicated that 'Florence' was more susceptible to *C. acutatum* than other strawberry cultivars, for instance, 'Darselect'. The susceptibility of various cv. was unequal.

A source of inoculum is also essential for disease development. The susceptibility of cv. is important for anthracnose development. Furthermore, the strawberry flower is more susceptible compared to immature fruit [13]. Jacobs et al. [39] found that there are no significant differences between *Colletotrichum* species, but a significant difference among strawberry genotypes. The cv. 'Elkat' and 'Deluxe' showed the highest susceptibility; however, 'Rumba' and 'Asia' had a lower disease severity index. Casado Diaz et al. [10] and Wagner et al. [6] observed that 'Camarosa' was very susceptible to *C. acutatum*. We observed a slight trend between soil and plant parts contamination with pathogenic fungi and susceptibility to *C. acutatum* in two strawberry cvs. The highest soil contamination of fungi was found in cv. 'Elkat', also cv. 'Elkat' was distinguished as the most susceptible to *C. acutatum* among all studied cv. The *Colletotrichum* spp. pathogen was most frequently found in cv. 'Malvina', soil and plant parts contamination by pathogenic fungi was also found of the highest levels, furthermore, cv. 'Malvina' showed high susceptibility to *C. acutatum.*

Temperature plays an essential role in *Colletotrichum* species complex development, spore germination and appressorial formation [17]. In the literature, it is considered that strawberry anthracnose is a warmer climate zone pathogen with an optimal temperature of 26.7–32 °C for *C. fragariae* and around 20 °C for *C. acutatum* [12–15,18]. We evaluated the influence of temperature on anthracnose mycelium development. Our data confirmed the results of Feil et al. [5] that *C. acutatum* mycelium was able to grow at 5 and 10 °C. He et al. [15] observed that colony growth was fastest in the temperature range of 25–28 °C; however, we found that the optimal temperature for *C. acutatum* development was between 20–25 °C.

5. Conclusions

This study confirmed that meteorological conditions play an essential role in pathogen development. Pathogens' adaptation to conditions that are not inherent in their growth and their spread poses increasing management challenges. The soil and the remains of plant parts are the primary sources of plant diseases.

The results of this study revealed that pathogen distribution in plant parts and soil varied depending on cultivar. For *C. acutatum*, a temperature of 20–25 °C is optimal for pathogen development. The monitoring of meteorological conditions, evaluation of inoculum source and the selection of less susceptible cultivars could reduce or avoid yield losses caused by *C. acutatum*. In future, due to climate change, *C. acutatum* occurrence and distribution will be affected.

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