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Foliar pathogens of sweet and sour cherry in Serbia

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Abstract: In recent years, sweet and sour cherry production in Serbia has increased. Under Serbian agroecological conditions, pathogens causing leaf diseases threaten the success of sweet and sour cherry production. In the period 2012–2019, the health status of cherries was monitored in more than 30 locations. Depending on the production system, the following leaf pathogens were identified: *Blumeriella jaapii*, *Wilsonomyces carpophilus*, *Mycosphaerella cerasella*, *Phoma prunicola*, *Podosphaera clandestina* and *Pseudomonas syringae* pv. *syringae* and *morsprunorum* race 1. Leaf pathogens caused premature defoliation, which adversely affected bud formation for the next growing season and increased susceptibility to freezing. Therefore, attention should be focused on correct leaf pathogen identification, and proper selection, application and rotation of fungicides.

Keywords: sweet cherry, sour cherry, leaf, disease, plant pathogenic fungi, bacteria.

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Introduction

Sweet cherry (*Prunus avium* L.) and sour cherry (*P. cerasus* L.) production is threatened by a significant number of phytopathogens affecting cherry leaves and causing premature defoliation, reduced shoot growth, increased susceptibility to winter injury, higher tree mortality, branch breakage and replanting problems.

Cherry leaf spot caused by *Blumeriella jaapii* (Rehm) Arx (syn. *Coccomyces hiemalis* Higgins; anamorph *Phloeosporella padi* (Lib.) Arx, syn. *Cylindrosporium padi* (Lib.) P. Karst. Ex Sacc.) is one of the most important and economically significant fungal pathogens affecting sweet and sour cherry both worldwide and in Serbia (Jones and Sutton, 1996; Schuster, 2004; Elmhirst, 2006; Lindhard Pedersen *et al*., 2012; Balaž *et al.*, 2012; Iličić *et al.*, 2017; 2018a). *Blumeriella jaapii* also affects nursery-grown stone fruits, as well as several cultivated ornamental and wild *Prunus* species (Jones, 1995). This pathogen primarily affects leaves, thus compromising photosynthetic ability, causing early defoliation, yield reduction and lower fruit quality. In some years, pathogen such as *Wilsonomyces carpophilus* (Lev.) Adaskaveg, Ogawa and Butler (previously named *Stigmina carpophila* (Lév.) M.B. Ellis (1959) – Coryneum blight, a causative agent of shot hole disease, can cause severe damage. The disease affects buds, shoots, leaves and fruits (Ogawa and English, 1995). Cercospora leaf spot (*Cercospora circumscissa* Sacc. syn. *C. cerasella* Sacc. teleomorph *Mycosphaerella cerasella* Aderhold) probably has worldwide prevalence. This pathogen has been detected on sweet and sour cherry and other *Prunus* species (Sztejnberg, 1986; 1995). In older orchards with minimal chemical treatments used, pathogens such as *Phoma prunicola* (Opiz.) Wr. & Hochapf. (syn. *Phyllosticta prunicola* Sacc., *Phoma pomorum* Thuem.) and *Podosphaera clandestina* (Wallr.: Fr.) Lév. can occur.

The bacterium *Pseudomonas syringae* can affect more than 180 plant species, including annual and perennial plants, fruit trees, ornamentals and vegetables (Agrios, 2005). Bacterial canker caused by *P. syringae* pathovars *syringae* and *morsprunorum* race 1 and 2 is one of the most serious diseases affecting stone fruit trees worldwide (Jones, 1971; Wimalajeewa and Flett, 1985; Vicente *et al.*, 2004; Bultreys and Kaluzna, 2010; Konavko *et al.*, 2014; Balaž *et al.*, 2016; Iličić *et al.*, 2017). This pathogen can attack all plant organs, including trunk, branches, leaves, fruits, flowers and buds, causing dieback. On leaves, bacterial canker pathogens cause circular lesions that fall out and produce "shot holes".

In the world fruit growing area, leaf pathogens such as *Taphrina cerasi* (Fuckel) Sadebeck, *Apiognomonia erythrostoma* (Pers.) and *Monilinia kusanoi* (Takahashi) Yamamoto were also described as leaf pathogens (Ogawa *et al*., 1995).

Disease development is mostly governed by certain weather parameters, such as temperature fluctuations, as well as averages of maximum and minimum temperatures, relative humidity and average of rainfall. If control measures are inadequate and conditions for leaf disease development favorable, infection will occur at much higher intensity, resulting in premature defoliation.

The aim of the present study was to determine the most common and potentially threatening leaf pathogens affecting sweet and sour cherry in the agro-ecological conditions of Serbia.

Material and method

Symptom observation and sample collection. In the period 2012−2019, monitoring of the health status of cherry plants was carried out in more than 30 locations in Serbia, including plantations, orchards with extensive and intensive production conditions, as well as cherry nurseries and wild cherry forms (Table 1).

Diseased leaves with different symptoms (spots, shot hole, powdery mildew) were collected during the spring and summer. Samples were placed in sealed plastic bags and stored in a refrigerator (4°C) for 2−3 days, until examination and/or pathogen isolation (Laboratory of Phytopathology, Faculty of Agriculture, University of Novi Sad).

Isolation. Diseased sweet and sour cherry leaves were first washed under tap water, dried on sterile filter paper and surface disinfected with alcohol. The isolation was performed on Potato Dextrose Agar (PDA), Water Agar (WA), Nutrient Agar (NA) and Nutrient Sucrose Agar (NSA). Small fragments taken from the margin of healthy and diseased tissue or formed stromata were placed onto PDA and WA for fungal isolation. For bacterial isolation, fragments were macerated in sterile distilled water (SDW), left for 20 minutes and plated on NA and NSA media. The Petri dishes were kept at 25 ± 1ºC for 2 to 21 days.

Diseased cherry leaves were also placed in a moist chamber for a few days for pathogen sporulation.

Identification. To identify the pathogens involved in cherry leaf diseases, standard phytopathological methods for fungal (Muntanola-Cvetković, 1987; Delibašić and Babović, 2006) and bacterial identification (Lelliott and Stead 1987; Lelliott *et al.* 1966; Latorre and Jones 1979; Schaad 2001) were used.

Pathogenicity tests. Healthy sweet and sour cherry leaves were artificially inoculated by spraying using the conidial conc. 10^7 CFU/ml prepared from sporulation on naturally infected leaves in SDW and bacterial suspension conc. 10^8 CFU/ml prepared from bacterial isolates in SDW. Control leaves were treated with distilled water. Inoculated material was kept in a plastic box on moisture filter paper at ambient temperature, until symptom development.

Table 1. Sweet and sour cherry localities covered by monitoring

Locality	Species	Production system	Examination period	Symptom type
Selenča	Sweet cherry	Intensive production	2012-2019	leaf spot; bacterial leaf spot and shot hole
Kać	Sweet and sour cherry		2012-2019	leaf spot; bacterial leaf spot and shot hole
Karavukovo	Sweet cherry		2019	leaf spot
Ljutovo	Sweet cherry		2014-2018	leaf spot; bacterial leaf spot and shot hole
Mikićevo	Sweet cherry		2014-2017	leaf spot; bacterial leaf spot and shot hole
Gornji Tavankut	Sweet and sour cherry		2012-2016	leaf spot; bacterial leaf spot and shot hole
Srbobran	Sweet and sour cherry		2018-2019	leaf spot
Čelarevo	Sweet cherry		2012-2014	leaf spot
Temerin	Sweet cherry		2015-2018	-
Sirig	Sweet and sour cherry		2012-2019	leaf spot
Bačka Palanka	Sweet cherry		2012-2019	leaf spot; bacterial leaf spot and shot hole
Šabac	Sweet and sour cherry		2016-2019	leaf spot
Irig	Sour cherry		2016-2019	-
Čerević	Sweet cherry		2015-2019	leaf spot
Žitorađa	Sweet cherry		2018	bacterial leaf spot and shot hole
Vinča	Sweet cherry		2016	leaf spot; bacterial leaf spot and shot hole
Rimski Šančevi	Sweet and sour cherry	Extensive production	2012-2019	leaf spot; shot hole; powdery mildew
Donji Tavankut	Sweet and sour cherry		2013-2016	leaf spot; shot hole
Čenej	Sweet and sour cherry		2015-2019	leaf spot; shot hole; powdery mildew
Bođani	Sweet and sour cherry		2014-2019	leaf spot; shot hole; powdery mildew
Vajska	Sweet and sour cherry		2017-2019	leaf spot; shot hole; powdery mildew
Novi Sad	Sweet cherry		2018-2019	leaf spot; shot hole
Deronje	Sweet cherry		2016-2019	leaf spot; shot hole
Bački Petrovac	Sweet and sour cherry		2017-2019	leaf spot; shot hole
Kovilj	Sweet and sour cherry		2017-2019	leaf spot; shot hole; powdery mildew
Bocke	Sweet and sour cherry		2016-2018	leaf spot; shot hole; powdery mildew
Krčedin	Sweet and sour cherry		2015-2017	leaf spot; shot hole
Ruma	Sweet cherry		2017-2019	leaf spot
Sremski Karlovci	Sweet cherry		2016-2019	leaf spot
Bukovac	Sweet and sour cherry		2016-2019	leaf spot; shot hole
Banstol	Sweet and sour cherry		2016-2019	leaf spot; shot hole; powdery mildew
Valjevo	Sweet cherry		2018-2019	leaf spot
Ritopek	Sweet and sour cherry		2012-2019	leaf spot; shot hole; powdery mildew

Kanjiža	Sweet and sour cherry	**Nursery production**	2013-2014	leaf spot; bacterial leaf spot and shot hole
Rimski Šančevi	Sweet and sour cherry		2012-2019	leaf spot; shot hole; powdery mildew
Fruška gora	*Prunus* spp.	**Wild cherry population**	2016-2019	leaf spot; powdery mildew

Results

Pathogen identification and distribution. During the study period (2012−2019), the intensity of foliar diseases of cherries in Serbia increased. Leaf spot and shot hole symptoms were manifested during the spring and summer, depending on the pathogen present and the system of production (Figure 1).

All fungal foliar pathogens proved extremely difficult to isolate on the mentioned nutrient media. Identification of fungal pathogens was performed from sporulation (microscopic characteristics) on leaves placed in a moist chamber. Bacterial pathogens were successfully isolated from the symptomatic leaves collected in May and June. Based on the obtained results, leaf pathogens affecting sweet and sour cherry leaves in Serbia were the phytopathogenic fungi *B. jaapii, W. carpophilus, M. cerasella, P. prunicola* and *P. clandestina* and the bacteria *Pseudomonas syringae* pvs. *syringae* and *morsprunorum* race 1 (Figure 1). Symptoms of mentioned leaf pathogens are shown in Figure 2. Compared to sour cherry, sweet cherry was more susceptible to all examined fungal and bacterial leaf pathogens.

Blumeriella jaapii. Cherry leaf spot caused by *B. jaapii* is still one of the most important fungal diseases affecting sweet and sour cherry leaves. This pathogen was identified in orchards receiving reduced, inadequate chemical treatment (intensive, extensive production) or on unsprayed trees. Its presence was also confirmed in sweet and sour cherry nursery production as well as on wild cherry forms. *B. jaapii* symptoms occurred in early summer on the upper surface of leaves, as tiny, red to purple circular spots that enlarged and turned red-brown to brown. In the spot center on the leaf underside, light pink to white masses from slightly concave eruptions (acervuli) emerged with hyaline, elongated, curved or flexuous, with one or two septe conidia, causing secondary infection. Diseased leaves turned yellow, fell off the tree and severely affected the trees, which defoliated by midsummer. Defoliation made the trees susceptible to winter frost, especially in the case of young trees. In diseased trees, weak fruit buds, reduced shoot growth and death of fruit spurs were also common.

Differences in cultivar susceptibility to this pathogen were also noted (unpublished data).

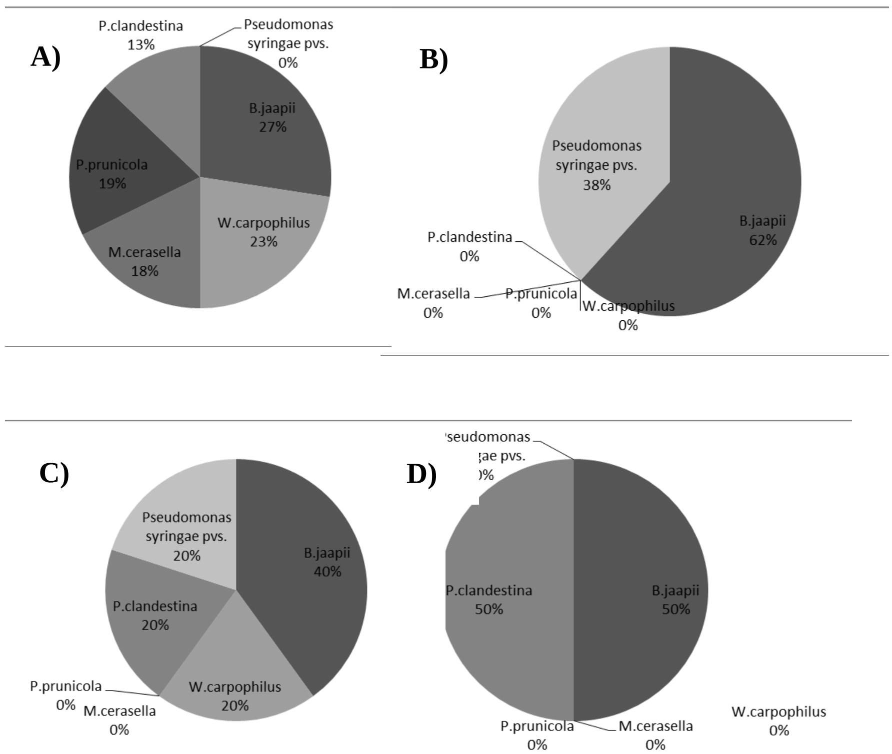

Figure 1. Distribution of cherry leaf pathogens in: A) extensive production; B) intensive production; C) nursery production; D) wild cherry population

Wilsonomyces carpophilus* - *S. carpophila. The causative agent of shot hole disease caused severe damage, especially when no dormant spray was applied (extensive and nursery production). Treatment with copper fungicides significantly reduced the amount of inoculum and prevented the occurrence of infections. In the present study, the first disease symptoms were observed on young leaves in the form of small red areas that expanded and became larger, irregular, reddish-brown to purple with a yellow halo border surrounding a white center. Spots dropped out, leaving a shot hole appearance, eventually causing the entire leaves to drop. The conidia of *S. carpophila* were ovoid, brown and dry, and were characterized by 2−5 thick dark brown cross walls (septa). In a few cases, spots on fruits sagged until the pit was revealed. *S. carpophila* disease symptoms are similar to those of bacterial leaf spot caused by *Pseudomonas syringae* pvs.

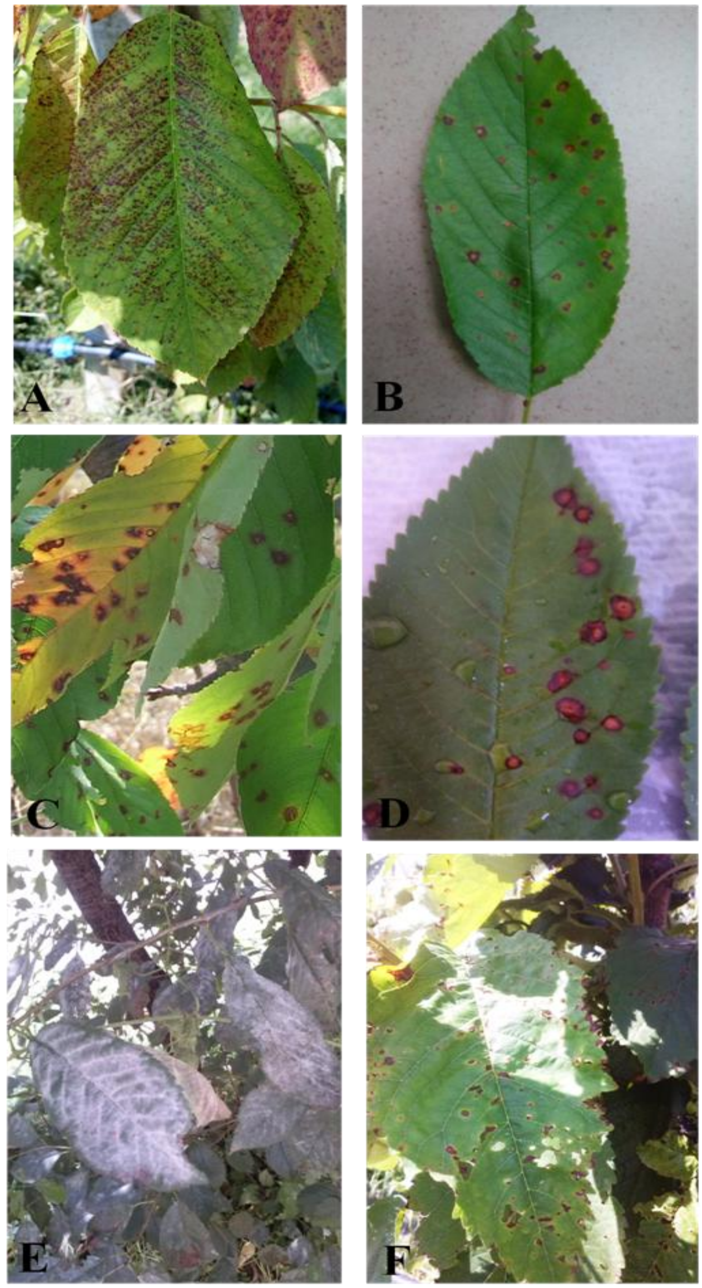

Figure 2. Symptoms on cherry leaves caused by: **A** - *Blumeriella jaapii*; **B** - *Wilsonomyces carpophilus;* **C** - *Mycosphaerella cerasella*; **D** - *Phoma prunicola;* **E** - *Podosphaera clandestina;* **F** - *Pseudomonas syringae* pvs. (Iličić R.)

Mycosphaerella cerasella* − *C. cerasella. Under extensive cherry production conditions, the presence of the pathogen *M. cerasella* − *C. cerasella* was detected. Disease symptoms emerged at the time of the occurrence of *B. jaapii*. Spots were initially round, reddish to brown and their size gradually increased, and the center turned light brown with brownish red edges. On a few occasions, spots coalesced into necrotic patches. Only the conidial stage was found; long, curved, olivaceous and septate conidia formed on brown, geniculate, flexuose conidiophores.

***Phoma prunicola*.** In early summer during the investigated period, the pathogen *P. prunicola* was also found in unsprayed orchards. Initially, spots were small, with a white center, but expanded rapidly and turned reddish to brown with brownish red edges (with a spot diameter of 4−8 mm). In the spot center, the pathogen formed pycnidia with pycnidiospores (single-celled, oval to elliptic).

Podosphaera clandestina. In some years, in extensive production, nursery production or on individual trees, in gardens and yards, as well as on wild cherry forms, the presence of powdery mildew on leaves caused by *P. clandestina* was noted. Circular white lesions (fungal mycelium and conidia−oidia) on the surface of young leaves were observed, while no symptoms emerged on fruits. In early summer, cleistothecia also developed.

***Pseudomonas syringae* pvs. (*syringae* and *morsprunorum* race 1).** In intensive (young) cherry plantations and orchards, in which control measures are adequate (timely and repeated fungicide applications), diseases caused by phytopathogenic fungi are usually not a problem, except in the case of *B. jaapii* (mid to late August). However, the two *P. syringae* pathovars (*syringae* and *morsprunorum* race 1) identified as part of the present study were problematic, as they are the causal agents of bacterial dieback and leaf spots in cherries. Spots and shot holes were observed on cherry leaves in May and June. Spots were initially water-soaked and then became brown-purple, round to angular, surrounded by yellow halos. Due to the development of numerous spots on leaves, centers of the necrotic spots dropped out, giving the leaf a shot hole appearance. Under favorable conditions (rainy and cold weather), the disease spread rapidly, often affecting the leaves of the entire crown.

Pathogenicity tests. To confirm that the studied pathogens caused symptoms on sweet and sour cherry, artificial leaf inoculation in laboratory conditions was performed. In the case of fungal leaf pathogens, the first symptoms occurred 7 to 10 days after inoculation, and all tested pathogens caused symptoms similar to those on naturally infected leaves. Bacterial leaf pathogens on the inoculated leaves caused symptoms after 3 to 4 days. Leaves inoculated with SDW were symptomless.

Discussion

In order to determine the most common and potentially threatening pathogens in sour and sweet cherry production in the agro-ecological conditions of Serbia, in this study we observed some major characteristics of the leaf pathogens determined.

The most destructive and most widely distributed foliar pathogen of sweet and sour cherry was *B. jaapii* (Table 1). The occurrence of this disease in intensive and extensive plantations was certainly caused by climate change (factors favoring sporulation, spore dispersal, germination and penetration), inadequate chemical control and increased susceptibility of sweet and sour cherry cultivars (Iličić *et al.,* 2017; 2018a). In intensive production, despite proper control practices, the presence of the pathogen was determined in mid to late August. This indicates that, despite the classical use of fungicides, great attention should be paid to the anti-resistance strategy related to

the combined application of fungicides with different mechanisms of action. It is also important to emphasize that the production of a large amount of secondary inoculum is also crucial for the severity of the disease (Outwater 2014). *B. jaapii* was an important pathogen for sweet and sour cherry nursery production; in young plants defoliation occurred in mid-summer, as reported earlier by Jones (1995) and Balaž *et al*. (2012). The fungal leaf pathogens *W. carpophilus, M. cerasella*, *P. prunicola* and *P. clandestine* were also recorded. They were present in orchards with minimized chemical control (extensive production), on unsprayed trees, in nurseries and on wild cherry forms. In intensive cherry plantations and orchards *P. syringae* pathovars causing bacterial leaf spot are becoming a major problem. Factors that contribute to the occurrence and spread of these pathogens are latently infected planting material, new cultivars, rootstocks, new technology of cherry production as well as the epiphytic nature of the pathogen (Iličić *et al*., 2018b).

To prevent all foliar diseases in cherries, it is recommended to apply two autumn sprays of copper-based fungicides to reduce the infectious potential and epiphytic population, and one spray in early spring, before bud break. Two to three pre-harvest and 3–5 post-harvest treatments are necessary, especially if spring and summer are rainy. For this purpose, fungicides based on captan, mancozeb, difenoconazole, tebuconazole, dodine, prochloraz, thiophanate methyl, dithianon and chlorothalonil are used to protect against fungal leaf pathogens.

All phytopathogens examined in the present study cause premature defoliation and reduce the number of formed buds and fruit yield in the following year. Moreover, the affected trees become more susceptible to frost and low winter temperatures, which increases tree mortality. Timely and correct pathogen identification is thus necessary for appropriate disease management.

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PATOGENI LISTA TREŠNJE I VIŠNJE U SRBIJI

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Rezime

Poslednjih godina proizvodnja trešnje i višnje u Srbiji je povećana. U našim agroekološkim uslovima, patogeni lista ugrožavaju proizvodnju ovih perspektivnih voćnih vrsta. U periodu 2012–2019. godine praćeno je zdravstveno stanje trešnje i višnje, na više od 30 lokaliteta. Zavisno od sistema proizvodnje identifikovani su sledeći patogeni lista: *Blumeriella jaapii, Wilsonomyces carpophilus, Mycosphaerella cerasella, Phoma prunicola, Podosphaera clandestina, Pseudomonas siringae* pv. *syringae* i *morsprunorum* rasa 1. Patogeni lista izazivaju prevremenu defolijaciju, što negativno utiče na formiranje pupoljaka za narednu vegetaciju i povećanu osetljivost na izmrzavanje. Stoga pažnju treba usmeriti na ispravnu identifikaciju patogena, pravilan izbor i rotaciju fungicida različitog mehanizma delovanja, kao i optimalno vreme za njihovu primenu.

Ključne reči: trešnja, višnja, list, bolest, fitopatogene gljive, bakterije.