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*XV International Scientific Agriculture Symposium
"Agrosym 2024"
Jahorina, October 10-13, 2024*

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MYCOPOPULATION ON RASPBERRIES IN SERBIA

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Abstract

Raspberry is a perennial deciduous plant, bushy or semi-shrubby, with annual and long biennial shoots, which belongs to the rose family. For many years, raspberries have been among the most economically important types of fruit in the Republic of Serbia. So far, there have been no systematic researches on raspberry mycopopulation in Serbia. In this paper, we present the results of preliminary research on raspberry mycopopulation originating from Serbia. A total of 28 randomly collected samples from three locations in Serbia were analyzed. Root and stem were analyzed. Plant fragments were carefully washed under running water. The samples thus prepared were disinfected with 1% sodium hypochlorite (NaOCl) for 1 minute and washed three times in sterile distilled water. They were then dried on sterile filter paper and placed on potato dextrose agar (KDA). In each Petri dish, five fragments taken per sample were placed in five repetitions, and incubated in a thermostat at 24°C. The examination was performed every three days, and on most samples mycelia developed around the plant fragment by the 14th day. Microscopic examination was performed using an Olympus CX31 microscope. The frequency of isolation was calculated in % according to the formula: number of colonized parts with fungi/total number of analyzed plant parts x 100. A total of 700 plant fragments were examined, from which six genera of fungi were isolated: *Didymella*, *Fusarium*, *Rhizoctonia*, *Verticillium*, *Alternaria* and *Epicoccum*.

Key words: *Frequency of isolation, mycopopulation, raspberry*

Introduction

Raspberry is a perennial deciduous plant, with bushy or semi-shrub growth, with annual and long biennial shoots, which belongs to the Rosaceae family. For many years, raspberries have been among the most economically important fruit species in the Republic of Serbia (Leposavić, 2023). The world production of raspberries in 2019 was 684,000 tons (Petrović *et al.*, 2020; Leposavić, 2023). On the European continent, Russia, Poland, Serbia and Spain are the biggest producers. However, the largest commodity production is realized in Serbia, because more than 95% of processed and fresh raspberry fruits are placed on the world market.

The most common causes of raspberry diseases are phytopathogenic fungi, bacteria and viruses. Due to the characteristic habitus of plants and intense vegetative growth, microclimatic conditions are created in raspberry plantations that favor the development of pathogens and the emergence of infections. Phytopathogenic fungi represent a very numerous and economically harmful group of pathogens on raspberries. The most economically important fungal diseases on raspberry are: *Didymella appianata* (Niessl.) Sacc. – spur blight; *Elsinoe veneta* (Burkh.) Jenkins – anthracnose (cane spot); *Phragmidium rubi-idaei* de Candolle P. Karsten – raspberry rust; *Paraconiothyrium fuckelii* (Saccardo) Verkley &

Gruyter – raspberry blight; *Phytophthora rubi* Man in't Veld – raspberry root rot; *Podosphaera macularis* (Wallroth) Braun & Takamatsu – powdery mildew; *Verticillium dahliae* Klebahn and *Verticillium albo-atrum* Reinke & Berthold – verticillium wilt; *Botrytis cinerea* Pers. Fr. (teleomorph *Botryotinia fuckeliana* (de Bary) Whetzel) – raspberry gray mold (Hai and Sutton, 1997; Munro *et al.*, 1988; Williamson, 2003; Williamson *et al.*, 2007; Mirković *et al.*, 2015). Among the bacterial disease-causing agents of raspberry, the most important are: *Agrobacterium tumefaciens* (Smith & Townsend) Conn – crown gall disease, *Pseudomonas syringae* pv. *syringae* van Hall. – pseudomonas blight disease and *Erwinia amylovora* (Burrill) Winslow, Broadhurst, Buchanan, Krumwiede, Rogers & Smith – fire blight (Braun and Hildebrand, 2006; Ivanović *et al.*, 2012). More than 30 viruses that can infect raspberries have been described in the world literature. Viral diseases occur everywhere where raspberries are grown. Economically the most important some viruses of raspberries are: black raspberry necrosis virus, BRNV; Raspberry leaf mottle virus, RLMV; Rubus yellow net virus, RYNV; raspberry bushy dwarf virus, RBDV; raspberry leaf blotch virus, RLBV and others (Jevremović and Paunović, 2011; Jevremović *et al.*, 2016; Jevremović *et al.*, 2019).

Considering the great importance of raspberry as a fruit species, especially for less developed hilly and mountainous areas in Serbia, the aim of this work was the isolation and morphological determination of mycopopulation on raspberry in order to better understand the problems in raspberry production (extinction of plants and reduction of yield) as a consequence of the presence of phytopathogenic fungi. Despite the intensive effort to find alternative solutions for controlling phytopathogenic fungi on raspberry, chemical protection is the dominant method. However, the application of chemical measures is difficult. The biggest problem is the timing of fungicide application, because flowering and raspberry harvest often overlap, and the presence of pesticide residues in fruits can be a problem. Also, the significant problem is the fact that a small number of fungicides are registered for the control of phytopathogenic fungi on raspberries.

Materials and methods

The samples were collected from 2019 to 2022 in the period June - August. A total of 28 samples from three localities in Serbia were analyzed (Čačak 8, Ivanjica 10, and Arilje 10). The experiment was conducted in the phytopathological laboratory of the Faculty of Agriculture in Kruševac, University of Niš. The plant parts were carefully washed under running water. After washing, parts of the stem and roots were cut into pieces measuring 0.5–1 cm. The samples thus prepared were disinfected with 96% ethanol for 30 seconds, 1% sodium hypochlorite (NaOCl) for 1 min and washed three times in sterile distilled water. They were then dried on sterile filter paper and placed on potato dextrose agar (PDA) with streptomycin. In each Petri dish, 5 plant parts were placed, in five repetitions. They were kept in a thermostat at 24°C and a light regime of 12 hours day/12 hours night. Examination was performed every 3 days, and on most samples, mycelia developed up to two weeks. The developed mycelia were screened on a new PDA substrate and, after the initial growth, the top part of the mycelium was screened again on PDA. Microscopic examination was performed using an Olympus CX31 microscope. Morphological identification of fungi was performed using standard keys (Dhingra and Sinclair, 1995). The frequency of isolation in % was calculated according to the formula (Vrandečić *et al.*, 2011):

$$(\%) \text{ Isolation frequency} = \frac{\text{Number of segments containing the fungal species}}{\text{Total number of segments used in the isolation}} \times 100$$

Results and discussion

A total of 700 plant parts were examined, from which 6 genera of fungi were isolated: *Didymella*, *Fusarium*, *Rhizoctonia*, *Verticillium*, *Alternaria* and *Epicoccum* (Table 1). Macroscopic symptoms of infection were clearly visible on the plants from which the fungi were isolated. On all plants from which the fungi were isolated, clearly expressed symptoms on the stems in the form of necrotic spots and lesions were observed. From those plants *Alternaria* fungi were isolated. In plants where purple-brown necrosis was observed on the stems, in the lower third of the stem, the fungi from the genus *Didymella* were isolated. Fungi from the genera *Fusarium* and *Rhizoctonia* were isolated from the plants with symptoms in the form of light to dark brown necrosis on the root system. Discoloration of the conducting tissue was also observed in some plants on the root system, and fungi from the genus *Verticillium* were isolated from those plants.

Table 1. Frequency of fungal isolation on raspberry

Location	Number of samples Plant part	Fungi species	(%) Isolation frequency
Čačak 1	25	<i>Alternaria</i> sp., <i>Fusarium</i> sp., <i>Didymella</i> sp., <i>Verticillium</i> sp.	15, 10, 25, 10
Čačak 2	25	<i>Didymella</i> sp., <i>Alternaria</i> sp., <i>Epicoccum</i> sp.	25, 10, 5
Čačak 3	25	<i>Alternaria</i> sp., <i>Didymella</i> sp., <i>Verticillium</i> sp.	25, 20, 40
Čačak 4	25	<i>Alternaria</i> sp., <i>Didymella</i> sp., <i>Verticillium</i> sp.	15, 35, 15
Čačak 5	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp., <i>Epicoccum</i> sp.	25, 10, 5, 5
Čačak 6	25	<i>Didymella</i> sp., <i>Verticillium</i> sp.	60, 10
Čačak 7	25	<i>Didymella</i> sp., <i>Alternaria</i> sp., <i>Epicoccum</i> sp.	40, 15, 5
Čačak 8	25	<i>Didymella</i> sp., <i>Rhizoctonia</i> sp.	35, 20
Ivanjica 1	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Epicoccum</i> sp.	20, 5, 5
Ivanjica 2	25	<i>Didymella</i> sp., <i>Alternaria</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp.	60, 20, 10, 5
Ivanjica 3	25	<i>Didymella</i> sp., <i>Epicoccum</i> sp.	30, 35
Ivanjica 4	25	<i>Didymella</i> sp., <i>Alternaria</i> sp., <i>Fusarium</i> sp.	15, 35, 5
Ivanjica 5	25	<i>Didymella</i> sp., <i>Epicoccum</i> sp., <i>Rhizoctonia</i> sp.	25, 5, 15
Ivanjica 6	25	<i>Didymella</i> sp., <i>Verticillium</i> sp.	25, 15
Ivanjica 7	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp.	35, 10, 5

Ivanjica 8	25	<i>Didymella</i> sp., <i>Epicoccum</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp.	35, 5, 20, 5
Ivanjica 9	25	<i>Didymella</i> sp., <i>Verticillium</i> sp., <i>Alternaria</i> sp., <i>Epicoccum</i> sp.	10, 15, 5, 5
Ivanjica 10	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp.	25, 10, 15
Arilje 1	25	<i>Didymella</i> sp., <i>Rhizoctonia</i> sp., <i>Alternaria</i> sp., <i>Epicoccum</i> sp.	25, 15, 5, 10
Arilje 2	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp.	25, 10, 10
Arilje 3	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Alternaria</i> sp., <i>Epicoccum</i> sp.	25, 10, 15, 15
Arilje 4	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp.	30, 5, 15
Arilje 5	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp.	15, 10, 5
Arilje 6	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp., <i>Alternaria</i> sp.	5, 10, 5, 10
Arilje 7	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp.	25, 10, 10
Arilje 8	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp., <i>Alternaria</i> sp.	30, 15, 20, 5
Arilje 9	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Alternaria</i> sp., <i>Epicoccum</i> sp.	30, 5, 10, 20
Arilje 10	25	<i>Didymella</i> sp., <i>Fusarium</i> sp., <i>Rhizoctonia</i> sp.	15, 20, 5

D. applanata is widespread in all raspberry growing regions in Europe, America, Africa, Asia and Australia (Mirković, 2018). In the Fraser Valley, British Columbia in the 1930s, several phytopathogenic fungi were isolated from infected raspberry plants: *Leptosphaeria coniothyrium* (*Coniothyrium fuckelii*), *Ilyonectria destructans* (*Cylindrocarpon radiclecola*), *Fusarium* spp., *Cylindrocladium* spp., *Pythium* spp., *R. solani*, *Rhizoctonia* spp. After artificial inoculations of healthy raspberry plants, obtained with fungal isolates, it was determined that all isolated species can cause necrosis on the roots of healthy plants (Berkeley, 1936). Raspberries are the third most popular berry in the United States and a growing specialty crop for both the wholesale industry. Postharvest susceptibility to gray mold (*B. cinerea*) drastically reduces the shelf life of this delicate fruit (Hai and Sutton, 1997; Williamson, 2003; Williamson *et al.*, 2007; Harshman *et al.*, 2014). *D. applanata* causes spur blight of raspberry was isolated from canes of diseased raspberries in a plantation in Novosibirsk, Russia (Shternshis *et al.*, 2006). *Alternaria tenuissima* has been reported on raspberries in China, in 2014 and 2015, as a leaf spot disease was observed on cultivated raspberry. The disease incidence averaged approximately 75%. Some small, circular, light brown spots appeared on the leaves in the early stage of disease development. The spots gradually enlarged and became round or irregular, dark brown, and developed concentric rings with dark brown margins normally surrounded by light yellow halos measuring 0.5 to 4.0 mm. Finally, the necrotic spots often coalesced to large blights of 11.0 to 15.0 mm. Severely infected leaves aged early and some fell off (Cong *et al.*, 2016). Raspberry cane disease is caused by various phytopathogenic fungi, including *Fusarium avenaceum* (*Fusarium* wilt), *D. applanata* (spur blight), *L. coniothyrium* (cane blight) and *B. cinerea* (cane *Botrytis*) with *F. avenaceum* being the major cause of cane diseases in Northern

Germany (Girichev *et al.*, 2018). Soilborne pathogens are a major concern to red raspberry and strawberry producers throughout the Pacific Northwest. Red stele, caused by *Phytophthora fragariae* var. *fragariae*, and raspberry root rot, caused by *P. fragariae* var. *rubi*, can cause serious economic losses in cool, wet production areas (Pinkerton *et al.*, 2002). Soilborne fungal pathogens (such as *Phytophthora* spp., *Verticillium* spp., *Rhizoctonia* spp., *Pythium* spp. and *Fusarium* spp.), lesion nematode (*Pratylenchus penetrans*) and weeds are common pests of Mexican strawberry and raspberry nurseries. *Phytophthora* root rot caused by *P. fragariae* var. *rubi* is the most serious root disease of red raspberries in many growing regions Mexico. Raspberry roots are also very sensitive to excessive moisture and lack of oxygen in the soil for extended periods of time. These conditions cause decay and root death. Best roots are produced in non-infested soils with good drainage (Lopez-Aranda *et al.*, 2016).

Conclusion

Growing raspberries is a very important and economically significant activity in Serbia. One of the most important factors that limits the profitability of raspberry cultivation are phytopathogenic fungi, the cause of economically important diseases. In rainy and humid conditions, diseases develop more intensively, and yields in the following year may be significantly reduced. The use of pesticides, i.e., fungicides, is the dominant method for phytopathogenic fungi control in raspberries. The reason why raspberry protection is based on the use of fungicides is the lack of raspberry varieties that show tolerance to economically significant disease agents. Such as *Didymella*, *Rhizoctonia*, *Verticillium*, *Phytophthora* and *Botrytis*. However, the implementation of these measures is difficult. The biggest problem is the limited time of pesticide application because the flowering and harvesting of raspberries can take a very long time, over 60 days. As a problem in the chemical protection of raspberries, a fairly poor range of fungicides is cited as a result of chemical companies' lack of interest in registering new active substances and formulations because the market is small and the registration of preparations is expensive. However, the application of chemical agents to protect raspberries from pathogenic fungi is the only measure that is necessary for profitable production. Without the application of all other available measures, primarily agrotechnical and mechanical, their full effect will be absent.

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