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Effectiveness of the biodegradable product FitoBotryfun in improving the fruit quality of 'Čačanska Bestrna' blackberry

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A B S T R A C T

Blackberry (*Rubus* subgen. *Rubus* Watson) cultivar 'Čačanska Bestrna' has long been the most commonly grown blackberry cultivar in the Republic of Serbia, owing to its high adaptability to respective agro-environmental conditions. In recent years, in order to increase the production of biologically valuable food, the fruit growing technology has been directed towards the concepts of integral and organic production. Blackberry grey mould (*Botrytis cinerea* Pers.) is an economically significant agent of the disease, which during the ripening phase reduces fruit yield by about 30% each year. In this regard, the efficacy of the preventive and curative influence of foliar applied FitoBotryfun was examined for two consecutive years. Incidence of grey mould (degree of infection), morphometric traits (fruit weight and dimensions), chemical traits (soluble solids content, total and inverted sugars, and sucrose content) and fruit mineral nutrient content [macro- (Ca, Mg) and microelements (Fe, Mn, Cu, Zn)] were recorded. A high variability was found in the degree of infection between the different disease management practices (organic and conventional) and significant differences were found in the contents of sugars, Ca, Mg and Fe in the fruit. The variations over the years were observed for the same quality traits, except for fruit height. The results obtained suggest that the foliar application of FitoBotryfun during the growing season can be effective in reducing losses in the quality of blackberry fruit during ripening as well as negative effects of conventional control methods on human health and the environment.

Keywords: grey mould, berry size, sugars, mineral elements, blackberry.

И З В О Д

Највише гајена сорта купине у Републици Србији је Чачанска бестрна због своје високе прилагођености агроеколошким условима. Последњих година, у циљу повећања производње биолошки вредне хране неопходно је и технологију гајења воћака усмеравати према концептима интегралне и органске производње. Сива трулеж плода (*Botrytis cinerea* Pers.) купине представља економски значајног проузроковача болести, који годишње, током фенофазе зрења, смањује приносе за око 30%. С тим у вези, током двогодишњег периода испитана је ефикасност превентивног и куративног деловања фолијарно примењеног производа FitoBotryfun. Испитивани су појава сиве трулежи плода (степен заразе), физичке особине (маса и димензије плода), хемијске особине (садржај растворљивих сувих материја, садржај укупних и инвертних шећера и садржај сахарозе), као и садржај минералних елемената [макро (Ca, Mg) и микроелемената (Fe, Mn, Cu, Zn)] у плоду. У различитим третманима заштите (конвенционални и органски) утврђене су значајне разлике у степену заразе, али и садржају шећера и Ca, Mg и Fe у плоду. Значајност разлика по годинама проучавања евидентирана је у погледу истих особина, изузев дужине плода. На основу добијених резултата може се закључити да фолијарна примена FitoBotryfun-а током вегетације може бити ефикасна мера којом ће се смањити губици у квалитету плода током зрења, као и штетан утицај конвенционалног програма заштите на људско здравље и животну средину.

Кључне речи: сива трулеж, крупноћа плода, шећери, минерални елементи, купина.

1. Introduction

Besides raspberry and strawberry, blackberry is also included in the group of economically important berries in Serbia (Nikolić and Milivojević, 2015). In the past decade, according to Strik et al. (2014), Serbia was among 4 world leading blackberry producers with 69% share of total European and 18% of total word production while according to the Republic Statistic Department of Republic of Serbia, the production increased 16% compared to previous period.

Blackberry plantings in Serbia are dominated by the cultivars 'Čačanska Bestrna' and 'Thornfree', with more than a 95% share, followed by 'Black Saten', 'Dirksen Thornless' and some more recent cultivars, such as 'Loch Ness', 'Chester Thornless', 'Triple Crown' (Nikolić et al., 2012). In order to achieve and maintain an optimal production level for the Republic of Serbia (25,000–30,000 t), as well as to eliminate variations in terms of yield, fruit quality, purchase price and market demand, the use of more recent growing technologies and processing methods as well as a partial change in cultivar choice are required (Karaklajić-Stajić et al., 2016).

A huge problem facing blackberry production is grey mould during the ripening phase caused by the phytopathogenic fungus *Botrytis cinerea* Pers., which reduces the yield of blackberry by about 30% each year (Tanović et al., 2009). On blackberry and raspberry, the pathogen causes progressive decay of infected fruit during the growing season, transport and storage (Tanović et al., 2008). Fresh fruits, including blackberry, demonstrate a considerable tendency to fungal infection during cultivation, harvest, post-harvest storage and marketing. Changes in the visual and compositional quality, and nutritive value of the fruit normally take place during storage (Ochmian et al., 2020). The phytopathogenic fungus *Botrytis cinerea* is commonly found on the fruit, in soil and on agricultural products and plant materials regardless of the climatic zone (Hole et al., 2005; Erisman et al., 2013). Studying the occurrence of fungal diseases in berry fruits, Tournas and Katsoudas (2006) found the highest incidence in the fruits of blackberry (78%), strawberry (77%) and raspberry (75%), while less infected fruits were found in blueberry (55%).

In order to overcome the aforementioned problem and produce food of greater biological value, it is necessary to intensify blackberry growing technology by introducing innovative disease management practices, which include the application of products that fight the economically important causal agents of diseases and satisfy basic criteria for organic production. On the other hand, organic agriculture has been on the rise and is attracting the attention of the food production sector in many parts of the world, since it revives eco-agricultural principles that are potentially more environmentally friendly and may provide products with few agrochemical residues (Azadi et al., 2011). Organic agriculture is defined today in various ways, although some common principles exist, such as not to use synthetic fertilisers and chemical pesticides (Lotter, 2003). In this regard, the application of substances that are permitted in organic production with the aim of preventive and curative action against grey mould have positive effects on the quality of blackberry fruit, since, as emphasised by Bielenin (2002), their foliar application needs to be carried out during the phenophase at a time of pathogen infection. Fruits are an integral part of the human diet as they supply vitamins and minerals, important constituents essential for human health (Akhtar et al., 2010).

There have been few studies and only a limited amount of published data which compare the influence of organic and conventional growing technologies on macro and microelement content in various food plants (Medvecký et al., 2014). The aim of this study is to provide information on the quality of blackberry (i.e. pomological, and morphometric fruit properties), cultivated using the foliar application of the natural product FitoBotryfun against *Botrytis cinerea* Pers.

2. Materials and methods

2.1. Plant material and experimental design

The investigation was conducted over a two-year period (2018–2019) in the experimental orchard of blackberry cultivar 'Čačanska Bestrna', which was established in 2006 at Gornja Gorevnica (43° 53'N latitude, 20° 20' E longitude, 290 m altitude) near Čačak, Western Serbia. This is mainly an upland area characterised by a temperate continental climate. The blackberries were planted in rows spaced 3.0 m apart with plants set at 1.5 m apart in the row, and trained to a three-wire trellis.

Weather conditions in Čačak were characterised by the mean growing season temperature of 16.3°C and total rainfall of 443.7 mm for the long-term averages (Table 1).

Table 1.
Mean monthly growing season temperatures and rainfall in Čačak in 2018 and 2019

Month	Air temperature (°C)			Rainfall (mm)		
	2018	2019	LTA*	2018	2019	LTA*
March	7.6	6.8	6.8	31.2	52.5	49.5
April	13.2	12.9	11.5	20.2	52.4	52.3
May	18.5	13.7	16.8	42.6	118.3	75.7
June	22.7	20.2	20.0	34.8	73.6	89.4
July	26.9	22.5	21.5	22.0	42.5	69.1
August	25.4	22.1	21.2	29.5	61.5	44.3
Mean of growing season	19.1	16.4	16.3	180.1	410.8	443.7

* Long-term average (54-year average, i.e. 1965–2019 period)

The soil in the blackberry plantings was vertisol, moderately supplied with organic matter (2.92%), and poor in N_{TOT} (0.11%), and soil pH in KCl 0.01 mol L^{-1} was 4.98. The contents of available soil P and K were 4.64 and 29.23 mg 100 g^{-1}, respectively. Under the agro-environmental conditions in the Republic of Serbia, blackberries have their best performance in warm pre-mountainous areas with high air humidity levels during the growing season, particularly at the fruit maturation stage (Nikolić and Milivojević, 2015).

The trial was conducted using a randomised block design. It included four replications of each treatment. The fertilisation and irrigation practices standard for the region were used.

2.2. Treatments

The study involved the application of the biodegradable product FitoBotryfun and the fungicide Switch 62,5 WG. Given the foliar product and fungicide, the trial involved two variants of disease management (5 bushes) in four replications with 40 plants. FitoBotryfun is an iron-based (2% Fe) natural product intended for preventive and curative action against grey mould in blackberries and has no waiting period.

Foliar treatments with FitoBotryfun and Switch 62,5 WG were applied three times during blackberry flowering: at the beginning of flowering (5–10% flowers open), during full flowering (7 days after the first treatment) and at the end of flowering (7 days after the second treatment) by an SR 420 motor sprayer (STIHL International GmbH Waiblingen, Germany) at a spray volume of 1 000 L ha^{-1} (Table 2). FitoBotryfun was used at a rate of 0.2% per treatment and Switch 62,5 WG at 0.8 kg ha^{-1} per treatment.

Table 2.
Foliar application dates during the research period (2018–2019)

Year	First treatment	Second treatment	Third treatment
2018	29st May	5th June	12th June
2019	28th May	3th June	10th June

2.3. Determination of the incidence of grey mould in blackberry

The incidence of grey mould in blackberries was established by counting infected and healthy fruits, from the beginning to the end of ripening (Table 3). The efficacy of the applied FitoBotryfun was determined based on the infection degree, which was calculated from the ratio of the number of infected fruits to total number of fruits, with values expressed in %.

Table 3.
Grey mould incidence

Disease management	Year	Dates of assessment of grey mould incidence								
Organic protection	2018	21st July	27th July	1st Aug	5th Aug	8th Aug	12th Aug	16th Aug	22nd Aug	/
	2019	16th July	20th July	24th July	27th July	31st July	3rd July	8th Aug	13th Aug	/
Conventional protection	2018	21st July	27th July	1st Aug	5th Aug	8th Aug	12th Aug	16th Aug	20th Aug	24th Aug
	2019	16th July	20th July	24th July	27th July	31st July	3rd Aug	8th Aug	14th Aug	/

2.4. Fruit quality analysis

Twenty-five fruits in each replication were randomly selected to determine average fruit weight using a METTLER balance (±0.01 g accuracy) and the data were expressed in g. Fruit dimensions (mm) (length, width and thickness) were determined by an inox vernier scale (±0.05 mm accuracy).

Blackberry fruit samples used for determining chemical traits and mineral content were collected from all the studied treatments in 2018 and 2019 at the commercial maturity stage. Fruits were randomly harvested from five bushes in four replicates ($n = 40$). Fruits were picked from different parts of the bushes to avoid fruit position effect.

Chemical parameters, viz. soluble solids content (SSC), total sugars (TS), reducing sugars (RS), titratable acidity (TA), and sucrose content (SC), were measured at the commercial maturity stage. SSC was determined by a digital refractometer (Carl Zeiss, Jena, Germany) at 20°C, and the data were expressed in %. TS (%) and RS (%) were determined on a fresh weight basis using the Luff-Schoorl method (Egan et. al., 1981). TA was measured by neutralisation to pH 8.1 with 0.1 N NaOH and the data were presented as % of malic acid. SC was calculated by multiplying the difference of the total and reducing sugars contents by the 0.95 coefficient.

The contents of macro (Ca and Mg) and microelements (Fe, Mn, Cu and Zn) were determined according to Morais *et al.* (2017) using atomic absorption spectrophotometry (Perkin-Elmer, PinAAcle 500, USA). Results of macro (Ca and Mg) and microelements (Cu, Mn, Zn and Fe) contents were expressed in mg kg^{-1} of fresh weight.

2.5. Statistical analysis

The data were presented as mean ± standard error (SE). Differences between means were compared by Duncan's Multiple Range test in a two-way analysis of variance (ANOVA) using the MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA). The significance of differences at a 5% level among means was determined significant.

3. Results and discussion

3.1. Incidence of grey mould in blackberries

The analysis of variance showed a significant effect of FitoBotryfun application on the number of infected fruits and degree of infection whereas year significantly affected the number of healthy and infected fruits and the degree of infection (Table 4). The effect of the disease management x year interaction showed significant differences between the number of infected fruits and the degree of infection.

Table 4.
Effects of FitoBotryfun application on the incidence of grey mould in 'Čačanska Bestrna' blackberries

Treatment	Total number of fruits	Number of healthy fruits	Number of infected fruits	Degree of infection (%)
Disease management (A)				
Organic protection	2,239.49 ± 57.33	2,116.28 ± 37.42	123.21 ± 14.24 b	5.50 ± 0.58 b
Conventional protection	2,327.48 ± 107.11	2,064.35 ± 97.13	263.13 ± 24.09 a	11.31 ± 1.16 a
Year (B)				
2018	2,175.14 ± 119.52	2,026,00 ± 98.04 a[1]	159.14 ± 26.18 b	7.32 ± 0.74 b
2019	2,225.84 ± 99.62	2,010.13 ± 74.88 b	215.71 ± 39.45 a	9.69 ± 1.43 a
ANOVA				
A	ns	ns	*[2]	*
B	ns	*	*	*
A × B	ns	ns	*	*

[1]Mean values followed by the different lower-case letters in columns represent significant differences.
[2]*: Indicates statistical significance at P ≤ 0.05, according to LSD test; ns:– non-significant.

The analysis of the results showed a larger total number of fruits in conventional protection treatments in 2019, but differences were not significant. The comparison of the efficacy of the preventive and curative action of foliarly applied FitoBotryfun and Switch 62,5 WG fungicide revealed a smaller number of infected fruits and a lower degree of infection in the former treatment compared to the latter treatment. The total number of fruits, number of healthy fruits, infected fruits and degree of infection ranged from 2,175.14 to 2,327.48, 2,010.13 to 2,116.28, 123.21 to 263.13 and 5.50 to 11.31, respectively.

The use of synthetic fungicides could prevent spoilage to some degree but some fungi could become resistant to commonly used pesticides (Spotts and Cervantes, 1986). Also, attempts to reduce the chemical contamination of the environment as well as health hazards associated with the consumption of pesticide residues dictate a reduction in the use of such chemicals (Tournas and Katsoudas, 2006). In terms of protection efficacy, positive effects of the preparation applied, i.e. a larger number of healthy fruits, less infected fruits as well as a lower infection rate, were observed. Across the study years, there was a significant variation in the number of healthy and infected fruits, and the degree of infection, which can be explained by different meteorological conditions in the two experimental years. Disease development is mostly governed by certain weather parameters, such as temperature fluctuations, as well as the averages of maximum and minimum temperatures, relative humidity and rainfall (Iličić et al., 2019).

A larger number of healthy fruits, a smaller number of infected fruits and, thus, a lower degree of pathogen infection were obtained in the first year. The foliar applied product FitoBotryfun had a positive effect in reducing grey mould incidence on fruits during the two-year period. Nieder et al. (2018) described efforts in the past decades to reduce toxicity and improve the effectiveness of pesticides through the use of new technologies and information, because this group of chemicals can severely affect animal and human health. On the other hand, the results obtained from this study could help growers to expand the application of organic product berry production and thus positively affect the minimal environmental pollution with improved quality of berry fruit.

3.2. Fruit quality parameters

The comparison of the different disease management methods showed that morphometric traits (fruit weight, height, width and thickness) were higher under organic protection, but these differences were not significant. Year had a non-significant effect on all the studied parameters, except fruit height. On the other hand, the effect of the disease management × year interaction led to statistically significant differences between the mean values of fruit weight and length (Table 5). The weight, height, width and thickness of the fruit ranged from 9.80 to 12.27 g, 25.33 to 55.74 mm, 21.69 to 24.19 mm and 20.66 to 26.76 mm, respectively.

Table 5.
Effects of FitoBotryfun application on the morphometric traits of 'Čačanska Bestrna' blackberries

Treatment	Fruit weight (*g*)	Dimensions of fruit		
		Height (*mm*)	Width (*mm*)	Thickness (*mm*)
Disease management (A)				
Organic protection	12.12±0.25	40.54±12.22	22.94±0.72	24.81±1.82
Conventional protection	9.98±0.40	30.69±0.80	21.92±0.37	21.31±0.66
Year (B)				
2018	11.07±0.58	43.21±11.59 a[1]	23.16±0.52	21.76±1.15
2019	11.03±0.59	28.02±1.52 b	21.69±0.76	24.36±1.72
ANOVA				
A	ns	ns	ns	ns
B	ns	*[2]	ns	ns
A × B	*	*	ns	ns

[1]Mean values followed by the different lower-case letters in columns represent significant differences.
[2]*: Indicates statistical significance at *P* ≤ 0.05, according to LSD test; ns:– non-significant.

All chemical properties of the cultivar 'Čačanska Bestrna', except SSC, when subjected to the analysis of variance, showed a significant effect of FitoBotryfun and year. Also, the interaction effect of variability factors significantly affected all the parameters, except SSC (Table 6). The values of the examined quality traits were higher in the organic management system than under conventional treatment.

The chemical traits subjected to the analysis of variance showed a significant effect of disease management and year on the contents of total and inverted sugars, and sucrose (Table 6). The significance of mean differences in all tested chemical parameters of the fruit, except SSC, was caused by the variability factor interaction (disease management × year).

Table 6.
Effects of FitoBotryfun application on the chemical traits of 'Čačanska Bestrna' blackberries

Treatment	SSC (%)	Sugars (%)		
		Total	Inverted	Sucrose
Disease management (A)				
Organic protection	11.55±0.53	4.93±0.11 a[1]	4.67±0.11 a	0.24±0.02 b
Conventional protection	10.70±0.56	4.57±0.01 b	4.11±0.04 b	0.41±0,03 a
Year (B)				
2018	11.47±0.72	4.87±0.14 a	4.56±0.16 a	0.30±0,02 b
2019	10.78±0.32	4.61±0.03 b	4.22±0.09 b	0.35±0,06 a
ANOVA				
A	ns	*[2]	*	*
B	ns	*	*	*
A × B	ns	*	*	*

[1]Mean values followed by the different lower-case letters in columns represent significant differences.
[2]*: Indicates statistical significance at $P \leq 0.05$, according to LSD test; ns:– non-significant.

Fresh soft fruits are highly perishable, and their quality and shelf-life can be greatly affected by different pre- and post-harvest factors and by plant stress.
These are the main factors contributing to the direct effect on marketability, and consumer acceptance, in addition to berry color which is determined by sugars, acidity and aroma compounds. In addition, there has recently been increasing interest in the content of other compounds with health-promoting properties in berries (Seeram, 2008). Cultivation and environmental factors, such as soil type, nutrient level and application strategy, have previously been shown to influence the nutrient supply to the plant and thereby affect plant concentrations and composition of all metabolites (Wang, 2006). Management practices which minimise fertiliser inputs and sustain good plant growth and yield without use of chemicals not certified as organic are therefore needed for continued expansion of organic production of blackberries (Harkins et al., 2013). It is important to devise a new fertiliser strategy in order to achieve and maintain an optimum balance between growth and productivity and obtain high nutritional quality of the fruit, and at the same time preserve the environment (Pešaković et al., 2020).

The foliar application of FitoBotryfun revealed significant differences in Ca, Mg and Fe content, while year had no statistically significant effect on the content of nutrients (Table 7). The significant influence of the disease management / year interaction on Ca and Fe content (Figures 1 and 2, respectively) were recorded.

Table 7.
Effects of FitoBotryfun application on the fruit nutrient content of 'Čačanska Bestrna' blackberries

Treatment		Fruit nutrient content (mg kg^{-1})					
		Macronutrients			Micronutrients		
		Ca	Mg	Fe	Mn	Cu	Zn
Disease management (A)	Organic protection	463.2±22.9 b[1]	326.0±19.2 b	15.38±6.23 a	9.13±0.44	0.98±0.07	1.32±0.08
	Conventional protection	597.9±12.6 a	386.1±8.01 a	1.98±0.18 b	12.95±0.37	0.72±0.01	1.50±0.04
Year (B)	2018	536.1±38.0	360.5±17.1	9.52±6.83	11.25±1.18	0.84±0.08	1.39±0.07
	2019	525.0±34.1	351.7±20.9	7.84±3.15	10.83±0.62	0.87±0.07	1.42±0.12
ANOVA							
A		*[2]	*	*	ns	ns	ns
B		ns	ns	ns	ns	ns	ns
A × B		*	ns	*	ns	ns	ns

[1]Mean values followed by the different lower-case letters in columns represent significant differences.
2*: Indicates statistical significance at $P \leq 0.05$, according to LSD test; ns:– non-significant.

Fe and Cu content in the cultivar 'Čačanska Bestrna' ranged from 1.98 to 15.38 mg kg^{-1} and 0.72 to 0.98 mg kg^{-1}, respectively, and were significantly higher in fruits treated with FitoBotryfun. In regard to the other studied nutrients (Ca, Mg, Mn and Zn), higher values were determined in conventional protection treatment. The contents of Ca, Mg, Mn and Zn ranged from 46.32 to 59.79 mg kg^{-1}, 32.60 to 38.61 mg kg^{-1}, 9.13 to 12.95 mg kg^{-1} and 1.32 to 1.50 mg kg^{-1}, respectively.

Figure 1. Content of Ca (A×B)

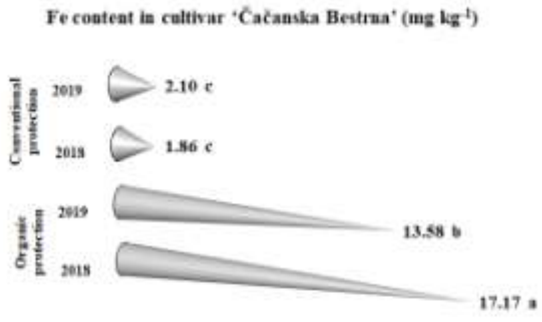

Figure 2. Content of Fe (A×B)

The effect of the disease management × year interaction (Fig. 1) inferred that Ca content in 'Čačanska Bestrna' was highest in bushes treated with FitoBotryfun during the first year, and lowest in the treatment with FitoBotryfun in the second year. On the other hand, Fe content in blackberries was highest in organic protection treatment in the first year and lowest in conventional protection treatment in the same year. During of examined period, FitoBotryfun had a stimulating effect on Fe content in blackberry.

The mineral contents in fruits are very dependent on the soil, fertilisation, climatic conditions and cultivar (Nour et al., 2011). In comparison to the literature data, the analysed fruits had lower concentrations of the minerals P, K, Ca, Mg and Zn and higher levels of Fe. In the organic protection treatment, an iron-based preparation was used, and therefore a higher iron content in the fruits of the tested blackberry cultivar was obtained. However, this difference is justified because, as already mentioned, we compared the composition of fruits receiving different protection treatments against grey mould i. e. conventional and organic ones. On the other hand, the values of the content of most nutrients in the blackberry fruit are in line with the results of Pereira et al. (2018), who evaluated the bioaccessibility of minerals from blackberries, raspberries, blueberries and strawberries. However, the contents of Ca and Mn were higher in organic protection treatment, which can be explained by a lower mobility of these elements.

4. Conclusions

The differences in the intensity of infection with the pathogenic fungus *B. cinerea* and the resulting quality of 'Čačanska Bestrna' blackberry under disease management treatments indicated that the organic product FytoBotryfun significantly contributed to improving fruit quality and reducing grey mould incidence accordingly. On the other hand, producers can provide guidance on the technology of cultivation and thus alleviate the problem that directly affects the quality of the 'Čačanska Bestrna' blackberry fruit and indirectly increase the health safety of blackberries by using an organic product for disease control.

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