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SCREENING FOR POLYPHENOL COMPOUNDS AND ANTIOXIDANT CAPACITY OF SWEET CHERRY FRUITS INFECTED WITH MONILINIA LAXA

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Summary: Monilinia laxa Aderh. and Ruhl. is the predominant causal agent of brown rot disease of stone fruit orchards, especially sweet cherries. The objective of this study was to identify reaction in response of nine genotypes cherry, with different pomological properties, against brown rot. These genotypes were harvested at commercial maturity from orchard in the Fruit Research Institute in Rimski Šančevi. The studied genotypes showed significant differences in terms of the occurrence of disease on fruits, both under artificial inoculation and infection in the field. Given the fact that sweet cherry fruits are prone to infection by a number of pathogens in the field, biochemical parameters were analysed on artificially inoculated fruits. Biochemical analysis of fruits determined significant differences in contents of total phenols, flavonoids and anthocyanins, as well as in antioxidant activity. It was genotype specificities and intensity of infection, as well as the interaction of the two that induced differences in the secondary biomolecules content and antioxidant activity. The majority of the genotypes examined showed high polyphenolics content, while under the infection, the content was significantly lower. Based on the results obtained, the secondary metabolites content can be used as one of the parameters for evaluating the resistance of sweet cherry genotypes to brown rot.

Key words sweet cherry genotypes, brown rot, polyphenol compounds, antioxidant capacity.

INTRODUCTION

Sweet cherry fruits contain various phenolic compounds, i.e. anthocyanins which contribute to the total antioxidant activity (Serrano et al., 2005). Different contents of polyphenolics governed by morphological features, stages of ripening or health status can be anticipated. Polyphenols are important for plants because they build an integral part of the cell wall structure. They primarily serve as polymers used as a mechanical barrier in the plant defence against microorganisms (Wallace and Fry, 1994; Strack, 1997). Additionally, under stress conditions, tissue damage or infection, plants inherently, as a defence mechanism, induce the synthesis of polyphenolic compounds (Britton, 1983; Dixon and Paiva, 1995). Three species of Monilinia genus can have a completely devastating effect on yield in seasons with favourable conditions for the development of the infection (Ogava et al., 1995; Hong et al., 1997; Larena et al. 2005). Monilinia laxa is the predominant causal agent of brown rot among sweet cherry fruits, and blossom and twig blight in stone fruit. It is believed that cracks on the cuticle, which account for 10% of fruit surface, are vital for the penetration and progress of the pathogen (Gibert et al., 2009). However, susceptibility of fruits to fruit cracking is decisive for possible infection therefore fruit rot occurs more commonly in genotype prone to fruit cracking (Holb, 2006). Typically, the pathogen penetrates into cracks on fruit skin however the infection may also develop on healthy fruits by contact with infected ones (Hrustić et al., 2012). The initial symptoms are manifested in small circular, brown, halo-like spots which develop around the site of infection, usually at sites of

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fruit injury. These injuries are most commonly due to insect activities which inflict damage on fruits thus allowing easy penetration of the pathogen inside the fruit. As the disease advances, the circular spot on the fruit expands and develops in only a few days on entire fruit in conditions of higher air temperature and humidity (Holb, 2008).

MATERIALS AND METHODS

Fruits of sweet cherry genotypes were collected in 2014. and 2015. from the productive orchard Fruit Research Institute in Rimski Šančevi (coordinates: 45°20´N, 19°51´E). Fruits of 9 genotypes (Priusadebnaja, Lionska rana, Junska rana, III/VAL, Merchant, Summit, Burlat (Bigarreau Burlat), Sue and Asenova rana), were included in this study. The classification of the sweet cherry genotypes according to the duration of fruit ripening was determined according to the average values for 2014 and 2015. 'Burlat' matures on May 20th, and this genotype was used as a standard. Four sweet cherry of them are early: Burlat, Lionska rana 2 days after Burlat, Asenova rana and Junska rana 5 days after Burlat. Three of them are medium–ripening: Merchant 7 days after Burlat, Priusadebnaja and III/VAL 12 days after Burlat. Two of them are late–ripening: Sue 17 days after Burlat, and Summit 14 days after Burlat. Genotypes studied differ in fruit skin color which varies from yellow to dark red. Yellow-skinned cherry with pink blush are: Priusadebnaja, Sue and Asenova rana. Red-skinned chery are: III/VAL, Burlat, Summit, Lionska rana, Merchant, Junska rana. Typically, flesh and juice colour are correlated with fruit skin color (Fogle, 1958).

A trial was conducted at the productive orchard Fruit Research Institute in Rimski Šančevi. The trial was designed according to randomized block system in 3 replicates. Thirty infected fruits, taken from two sweet cherry genotypes, two cherry trees each, were examined over the period of their respective ripening times. The first evaluation (May 20th) of brown rot was done by estimation of total sweet cherry fruit area infection per tree. Evaulation of the disease intensity on fruits was done during May and June in botanical maturity by the same scale. The intensity of infection was assessed from 0 to 4 (0-100 %). Fruits with no symptoms of infection were rated 0, whereas fully infected fruits were rated 4. Fruits with symptoms of infection induced by Monilinia laxa were collected from a sweet cherry (Prunus avium L.) planting established with 9 cultivars. The isolation of the pathogen was performed on collected samples using standard phytopathological methods (Dhingra and Sinclair, 1995). The isolate M3B5 was used for the artificial inoculation of sweet cherry fruits. After disease assessment, fruits were divided into two groups: healthy and infected.

In each groups content of polyphenols, flavonoids and anthocyanins as well as antioxidative activity was measured in three replication per cultivar.

Plant material for biochemical analyses. Dry plant material (DW) for polyphenol compouns and fresh plant material (FW) for antioxidant assays (1 g per sample) was ground to a fine powder and extracted with 70% aqueous acetone solution (50 mL) by sonication for 20 minutes in an ultrasonic bath at ambient temperature. The extracts were rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated until assayed.

Total polyphenols were determined spectrophotometrically (Jenway 6505, UK) by Folin-Ciocalteu procedure (Kroyer, 2004). Gallic acid (GAE) was used as a standard (covering the concentration range between 0.1 and 1.0 mg/mL) and results were expressed as miligrams of gallic acid (GAE) per gram of dry plant material (DW). The absorbance was read at 720 nm using a spectrophotometer.

Total anthocyanins were determined according to the pH differential spectroscopic method (Cheng and Breen, 1991). The content of total anthocyanins was expressed as milligrams of cyanidin 3- glucoside (C3G) equivalents per gram of DW. Extracts were diluted in 5 ml of two different buffers. After incubation at absorption (A) was measured at 510 and 700 nm.

Total flavonoids were determined according to the method described by Markham (1989). The amount of flavonoids was calculated as a rutin equivalent from the calibration curve of rutin standard solutions (concentration range between 0.1 and 1.0 mg/mL) and expressed as miligrams of rutin per gram of DW. Readings of the colored product were then taken at 430 nm.

The potential antioxidant activity of the test samples have been assessed based on scavenging activity of the 70% aqueous acetone sweet cherry extracts of the stable 1,1`- diphenyl-2-picrylhydrazyl (DPPH) free radicals (ABE et al., 1998). DPPH-radical scavenging activity was expressed as % of neutralized free radicals, assuming that the sample with the higher percentage has higher scavenging capacity. The absorbance was measured using a spectrophotometer at 517 nm. The FRAP assay was done according to Benzie and Strain (1996) with some modifications. Readings of the colored product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The standard curve was prepared using trolox. Results are expressed in mg TE/g FW. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve.

Statistical analyses. Results were expressed as mean of determinations of 3 independent samples made in triplicates. Statistical significance was tested by analysis of variance followed by comparison of means by Fisher

LSD test (P<0.05) calculated using STATISTICA for Windows version 12 (StatSoft, Inc., USA). Values for sweet cherry infection were analysed by non parametric statistics Kruskal-Wallis test.

RESULTS AND DISSCUSION

The studies of researchers showed that the chemical composition of the fruit is governed by genetic and environmental factors, technology of production, storage conditions (Borguini and Da Silva Torres, 2009). Sweet cherry genotypes respond differently to fruit rot, whereby fruits with thicker fruit skin are less sensitive to those with thinner one (Holb, 2006). Variety selection, breeding of less susceptible varieties, reduce the risk of disease incidence (Brown and Wilcox, 1989). Sweet cherry genotypes studied displayed different susceptibility to pathogens both under the artificial inoculation and natural infection. In order to have the response of a cultivar to infection on a biochemical level, the analysis of the polyphenolic components and antioxidant capacity was conducted.

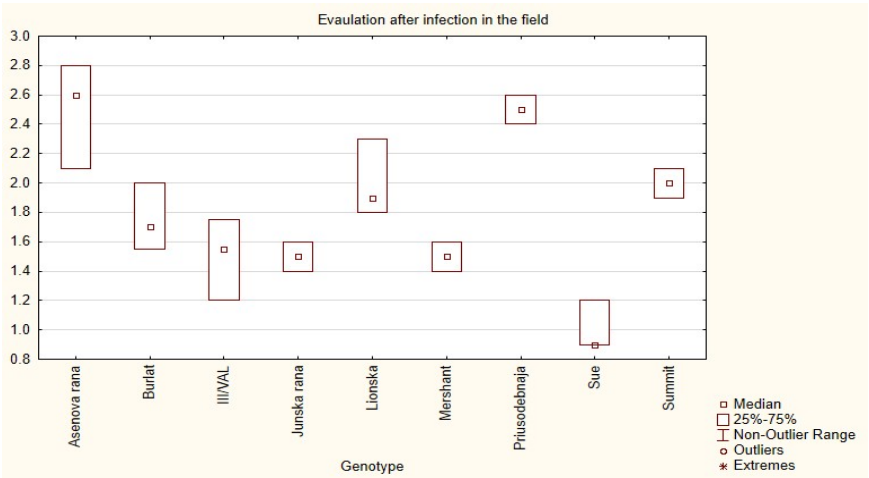

Figure 1. Evaluation on intensity of infection of Monilinia laxa fruits spot disease of sweet cherry genotypes influence of natural infection

Under the conditions of natural infection, the results pointed to different grades (Figure 1), which was anticipated, given the genotypes specificities and different fruit ripening times. The highest intensity of infection was observed in early ripening cultivars, i.e. 'Priusadebnaja' and 'Asenova Rana', with the highest infection rate (2.5) and 'Lionska Rana' and 'Summit' (2). Fruit flesh was softer in early ripening sweet cherry cultivars (Iezzoni et al., 1991). Fruit firmness was correlated with fruit ripening time (Fogle, 1961). It was found that sweet cherry genotypes with thicker cuticle were less susceptible to fruit cracking and fruit rot accordingly (Demirsoy and Demirsoy, 2004). The lowest infection rate was found in 'Sue' which was graded (1).

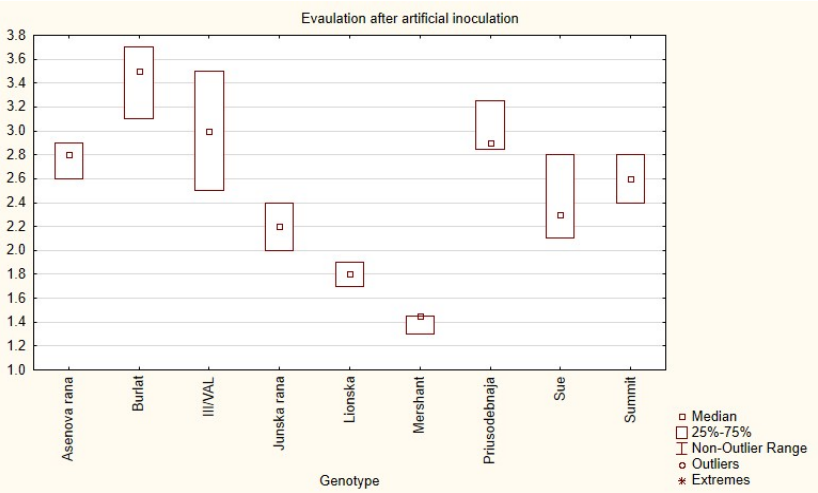

Figure 2. Evaluation on intensity of infection of Monilinia laxa fruits spot disease of sweet cherry genotypes after artificial inoculation

Fruits with the damaged skin are susceptible to Monilina laxa (Holb, 2008). Given that the third day after the artificial inoculation healthy and infected parts of the fruit were clearly distinguished, the assessment performed three days after the inoculation was taken as a parameter for determining the correlation between biochemical properties (Figure 2). There were statistically significant differences among the genotypes. The intensity of infection was lowest in 'Merchant' (value 1.4). Surprisingly, the highest intensity of infection was found in 'Burlat', graded with (3.4) given the fact that this genotype was singled out as a potential parent and the donor of genes of resistance to Monilinia laxa (Iezonni et al., 1991). Genotype 'III/VAL' and 'Priusadebnaja' also displayed high intensity of infection.

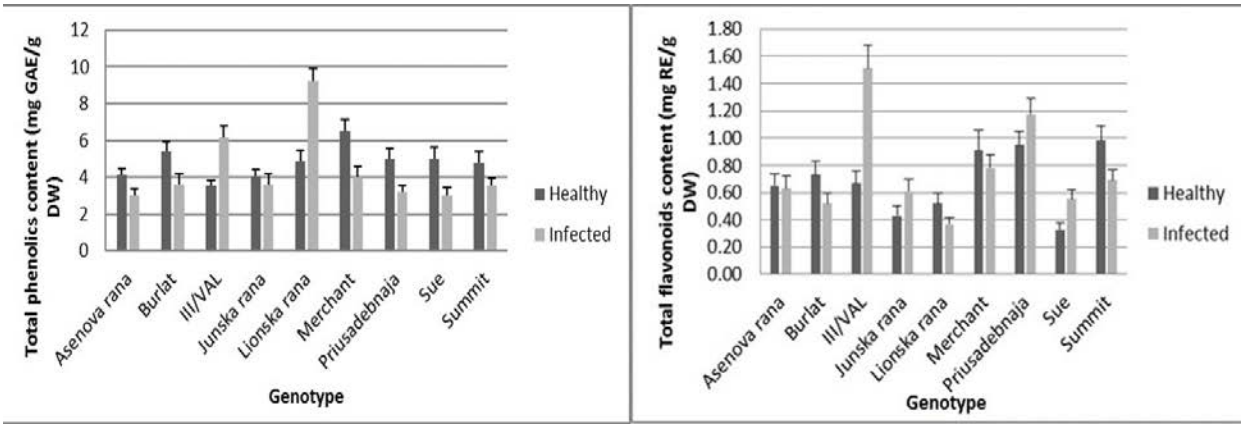

Figure 3. Total phenolics (TP) and total flavonoids (TF) content in healthy and sweet cherry fruits infected with Monilinia laxa. Bars=LSD 5%

The TP content in tested genotypes ranged from 3.53-6.50 for healthy fruits and 3.03-9.02 mg GAE/g DW for infected fruits (Figure 3). Two genotypes showed significantly higher TP content in infected fruits. It was founded in genotypes III/VAL and Lionska rana. Accumulation of phenolic compounds in the kiwi fruit infected by Botritis cinerea was reported by Wurms (2005). Resistance to Monilinia fructicola in peach Prunus persica L. was associated with a number of factors that included increased levels of phenolic compounds and other biomolecules (Gradziel et al., 2003). Seven other genotypes showed the opposite response, while wheat grown under pathogenic conditions responded similarly. In infected leaves influence of pathogen Ustilago tritici was found 31.75% to be lower to that of healthy ones (Tehmina et al., 2012). The amount of phenolics started to decrease with the progress of disease, influence of Alternaria triticina (Tyagi et al., 1998). The TF content varied from 0.33 to 0.98 mg RE/g DW for healthy fruits, while in the infected fruits the content of TF was from 0.37 to 1.51 mg RE/ DW (Figure 3). In four out of nine tested genotypes significant difference was found in TF content between healthy and infected fruits. For sweet cherry genotype Asenova rana, there was no significant difference in average TF content on the basic of the infection of this pathogen fungi. Genotypes Sue, Priusadebnaja III/ VAL and Junska rana contained higher amount of TF in infected fruits than in healthy fruits. 'Sue', which has low fruit cracking index, serves as the donor of genes for resistance to fruit cracking (Iezzoni et al., 1991; Sansavini and Lugli, 2008). It was reported that the decrease in concentration of some phenolic compounds in the epidermis of peach fruits correlated with a corresponding increase in disease susceptibility to Monilinia fructicola Wint. (Bostock et al., 1999).

The anthocyanins content of sweet cherry fruits ranged from 0.11-0.95 mg C3G equivalents per g DW (Figure 4). The highest TA content was in the healthy sweet cherry fruit III/VAL (0.95 mg C3G/g DW), followed by the Lionska rana (0.67 mg C3G/g DW) and Merchant (0.46 mg C3G/g DW). The lowest content of TA compounds was recorded in the healthy sweet cherry fruit Priusadebnaja (0.11 mg C3G/g DW). The total anthocyanins content was higher in fruits of genotypes with dark fruit skin. In most genotypes, the total anthocyanins were lower in infected fruits, except for Junska Rana, Priusadebnaja and Sue where the content was higher in infected fruits. Studies have shown that anthocyanins contents of sweet cherry genotypes were in the range of 0.35-0.69 mg C3G equivalents per g DW basis (Prvulović et al., 2011).

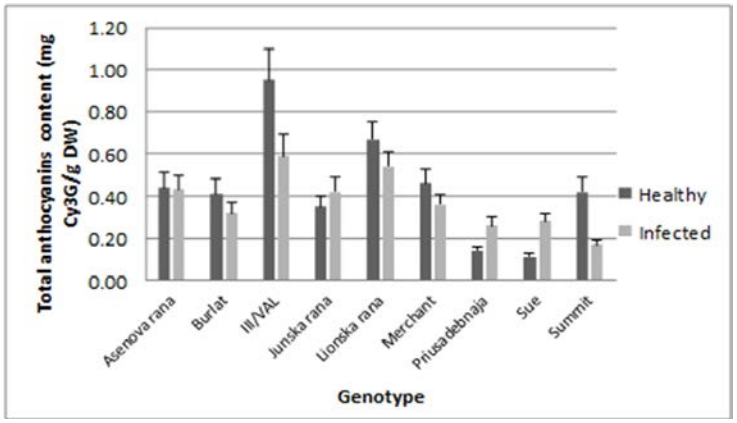

Figure 4. Total antocyanins (TA) content in healthy and sweet cheery fruit infected with Monilinia laxa. Bars=LSD 5%

The ability of the extract to reduce in vitro complexly-linked Fe3+ to Fe2+ presents the reducing capacity of extracts. Researchers introduced an approximation whereby the reduction capacity equals the antioxidant capacity of the extract (Benzie and Strain, 1999). The values of FRAP test suggested the higher reduction capacity in acetone extracts of healthy cherry fruits than of the infected ones (Figure 5). FRAP values were ranged from 15.48 to 33.69 mg TE/g FW for healthy and 14.72 to 28.68 mg TE/g FW for infected fruits. 'Sue' and 'Priusadebnaja' were the sweet cherries which exhibited greater reduction capacity under infection conditions.

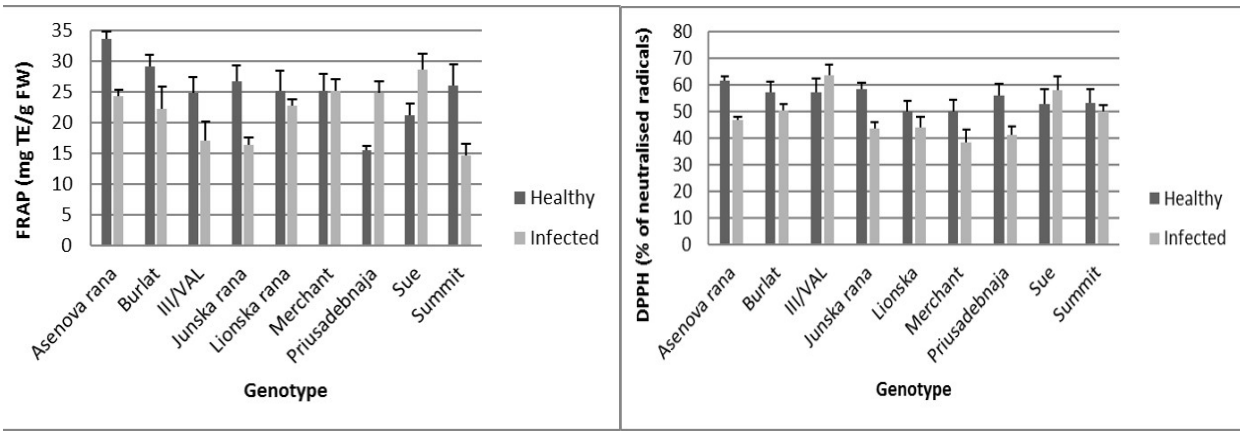

Figure 5. FRAP and DPPH activity in healthy and sweet cherry fruits infected with with Monilinia laxa. Bars=LSD 5%

The DPPH method provided an easy and rapid way to determine the antioxidant activity of healthy and infected cherry fruits tested in this study. Two out of nine tested cultivars had antioxidant activity higher in infected than in healthy fruits (Figure 5). The highest antioxidant activity was in the infected sweet cherry fruit III/VAL (63.53% of neutralised radicals). Other cultivars showed higher antioxidant activity in healthy fruits. The lower antioxidant activity was showed in infected fruit of Merchant (38.55% of neutralized radicals). The percent DPPH radical scavenging activity of cherry fruit recorded at un-ripened (44.32%), semiripened (53.83%) and at fully-ripened stage (72.99%) (Mahmood et al., 2013). Researchers determined that, in the presence of pathogens, the metabolism of a polyphenol was induced however the antioxidant capacity was reduced (Kiprovski et al., 2014).

CONCLUSION

Sweet cherry is a rich source of various phytochemicals. Both under the infection in the field and under artificial inoculation, the cherries studied exhibited statistically significant differences in the studied parameters. At the biochemical level, in the majority of sweet cherry genotypes infected with Monilinia laxa phenolics content and antioxidant capacity were lower. Polyphenols content and antioxidant capacity varies among genotypes, in both healthy and infected fruits. Some genotypes showed higher polyphenols content under infection. The parameters

studied can be used as a method of implementation of assessment of resistance to brown rot among sweet cherry cultivars.

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