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Postharvest changes in primary and secondary metabolites of sweet cherry cultivars induced by *Monilinia laxa*

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ABSTRACT

The aim of the study was to evaluate in which way nine cultivars of sweet cherry fruit cope with postharvest, artificial inoculation with *Monilinia laxa* pathogenic fungus through changes in sugars, organic acids and polyphenolic compounds, as well as which phenolics compounds are crucial participants in coping with the disease. Contents of sugars in this study were (g kg^{-1} dry weight): glucose 205–439 and 268–443, fructose 175–398.9 and 208.6–365.8 and sucrose 20–47.6 and 19.2–38.6 in healthy and infected fruit, respectively. Organic acids detected in sweet cherry cultivars were citric, malic, quinic, shikimic and fumaric acid and their content varied depending on cultivar and treatment. Only 'Burlat' accumulated tartaric acid after the infection. Of all phenolic acids, which content decreased in infected fruit, it is clear that only the more tolerant genotypes 'Merchant', 'Lionska', and 'Sue' enhanced content of 3-feruloylquinic acid (in healthy up to 93 and in infected fruit 105.3–139.1 mg kg^{-1}). The most abundant anthocyanins were cyanidin derivatives (80–266.1 and 10–3700 mg kg^{-1} in healthy and infected fruit, respectively). 'Burlat', 'Junska rana', Merchant' and 'Summit' dominantly had cyanidin-glucoside, while only infected fruit of 'Priusadebnaja' and 'Asenova rana' accumulated cyanidin-glucoside. 'Priusadebnaja', 'Asenova rana' and 'Lionska' had cyanidin-rutinoside. 'Lionska', 'Sue' and 'Asenova rana' had higher cyanidin and peonidin derivative contents in infected fruit (1.2 to 3-fold higher). Peonidine derivatives (peonidin glucoside and rutinoside), 5-carboxypyrano-cyanidin-rutinoside content mostly decreased after the infection (14.9–97.7 %). Bearing in mind obtained results, 3-feruloylquinic acid, flavonols and anthocyanins could be proposed as crucial participants in coping with the disease.

1. Introduction

Monilia laxa Aderh. and Ruhl. represents one of major pre- and postharvest pathogens of stone fruit, causing significant economic losses (Papavasileiou et al., 2015; Romanazzi et al., 2008; Hrustić et al., 2015). Airborne conidia, landing on green fruit in the orchard, may germinate and penetrate fruit skin, but they do not proceed further. The quiescent infections only become active as the fruit ripens and provide abundant conidia for infections. Infections commonly develop in mechanical wounds (Kader, 2002). Fruit rot starts with a small and round brown spots, which expands to entire fruit. Infected, aborted fruit remains in the tree and provides an additional source of infection (Nasrollanejad and Ghasemnezhad, 2009).

Plants have evolved complex, integrated defence system against potential pathogenic microorganisms. These mechanisms include preformed physical and chemical barriers as well as inducible defences, such as strengthening of the cell wall and activation of several biochemical pathways for the synthesis of different compounds (Ramonell and Somerville, 2002; Thatcher et al., 2005). According to Schovánková and Opatová (2011) apples inoculated with *M. fructigena* had different response to the infection in the fruit peel and flesh, accumulating total polyphenols in the healthy pulp in contrast to the area surrounding the rotten part.

Fruits and sweet cherry is a good source of phenolic compounds, sugars and organic acids (Ercisli et al., 2008; Usenik et al., 2008; Ercisli et al., 2012; Caliskan et al., 2017; Cuce and Sokmen, 2017). Their composition and concentrations largely affect the taste characteristics and organoleptic quality of fruit, colour and sensory properties such as bitterness and astringency (Kim et al., 2005; Serrano et al., 2005; Fazzari et al., 2008; Papp et al., 2010). Also, polyphenols contribute to the total antioxidant activity of plants (Kaur and Kapoor, 2001). Different contents of polyphenolics, sugars and acids governed by

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morphological features, stages of ripening or health status can be anticipated (Serrano et al., 2005).

Sweet cherry cultivars respond differently to fruit rot, whereby cultivars with thicker fruit skin are less sensitive to those with thinner one (Holb, 2006). However, susceptibility of fruit to cracking is decisive for possible infection therefore fruit rot occurs more commonly in cultivars prone to fruit cracking (Holb, 2006). Typically, the pathogen penetrates into cracks on fruit skin however the infection may also develop on healthy fruit by contact with infected ones (Hrustić, 2013). Three species of *Monilinia* genus can have a completely devastating effect on yield in seasons with favourable conditions for the development of the infection (Ogawa et al., 1995; Hong et al., 1997; Larena et al., 2005). *Monilinia laxa* is the predominant causal agent of brown rot among sweet cherry fruit, and blossom and twig blight in stone fruit. In Serbia, 96.34% of all diseased fruit are infected with *Monilinia* genus, while the other species of the genus occur less frequently, *i.e. M. fructigena* and *M. fructicola* (2.44 and 1.22%, respectively) (Hrustić, 2013). Cracks on the cuticle, which represents 10% of fruit surface, are vital for the penetration and progress of the pathogen (Gibert et al., 2009). It generally occurs several weeks before harvest, when fruit are the largest. The initial symptoms are manifested in small circular, brown, halo-like spots which develop around the site of infection, usually at sites of fruit injury. These injuries are most commonly due to insect activities which inflict damage on fruit 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).

The objective of the study was to evaluate response of nine cultivars of sweet cherry fruit to artificial inoculation with *Monilinia laxa* pathogenic fungus through changes in primary and secondary metabolism. Consequently, sugars, organic acids and polyphenolic compounds of both non-inoculated (healthy) and inoculated sweet cherry fruit infected with were identified and quantified. These results could explain in which way this pathogen affects postharvest quality of fruit of sweet cherry cultivars and which phenolics compounds are crucial participants in coping with the disease.

2. Material and methods

2.1. Isolation and characteristics of the pathogen

Diseased fruit, infected with *M. laxa,* were harvested for isolation and determination of the pathogen. The isolation of the pathogen (*M. laxa*) was performed from collected samples using standard phytopathological methods (Dhingra and Sinclair, 1995). Small fragments from the sites of contact of healthy and diseased tissues were cut off using a sterile scalpel, surface-sterilized in 2% sodium hypochlorite for 3 min and transferred onto a sterile PDA (Potato Dextrose Agar) medium (Muntañola-Cvetković, 1987). The media with pathogen were incubated in the dark at 24 °C until the incidence of fungal colonies. Following the incidence of mycelium (2–3 d after the isolation), fragments taken from the perimeter of the colonies were transferred on the PDA to obtain pure cultures. The resulting isolates were then subcultured into tubes containing inclined PDA medium and, subsequent to the development of the culture at 24 °C, were transferred and refrigerated at 4 °C (Dhingra and Sinclair, 1995). Obtaining 18 *Monilinia* spp.-like isolates was followed by the identification of isolates up to the species level, based on the study of pathogen and morphological characteristics. To test pathogenicity, specific morphological features (colony colour, edge features and form of the colony) were studied based on features described by Lane (2002). The examination of morphological characteristics involved the study of some macroscopic and microscopic characteristics of the selected pathogen isolates. Among the microscopic properties of the selected isolates, it was conidia form that was examined in the study. All the isolates grown on PDA medium developed light brown to gray rosette-shaped colonies, lobed edges and had no spores. Morphological characteristics above point to *M. laxa* species (Fig. 1A). The study of microscopic properties and the determination of the shape of conidia indicated that the selected isolates of *M. laxa* develop hyaline, single-celled conidia 7 d after growing on the medium. Conidia are circular to oval, unicellular, typically developed by *M. laxa* (Fig. 1 B,C). Obtained isolate (M3B5) was used for the artificial inoculation of healthy sweet cherry fruit.

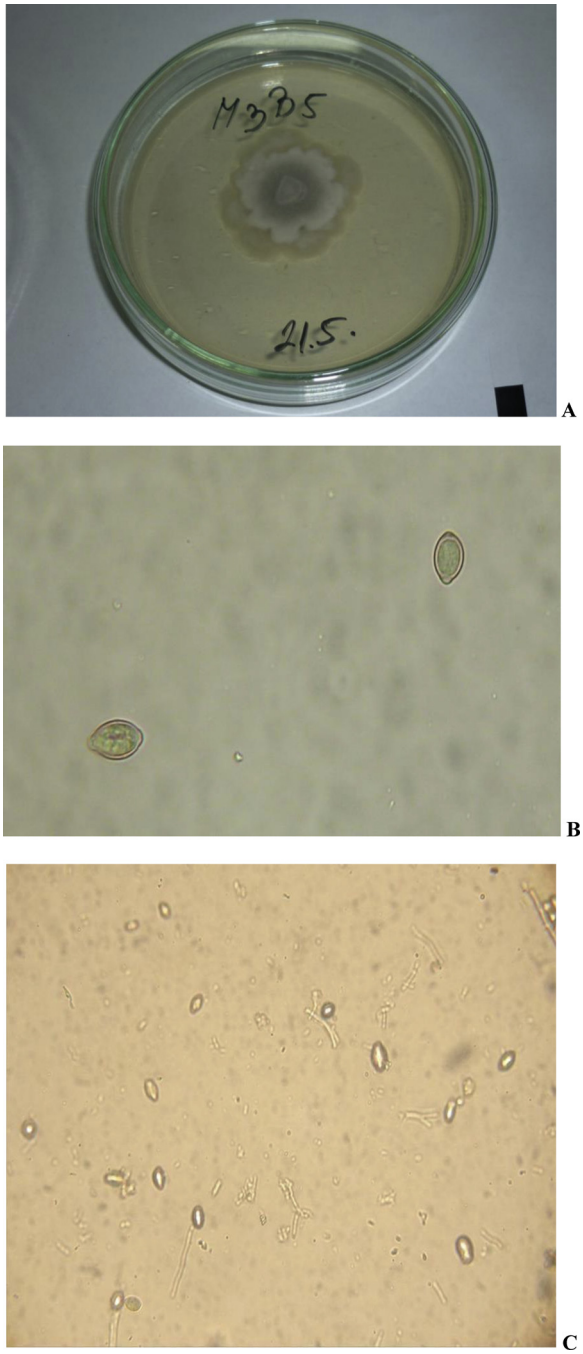

Fig. 1. *Monilinia laxa* isolate M3B5 on a PDA medium (A), BTC microscope view 10 × 40 (B) and 10 × 25 (C).

2.2. Plant material

Since the incidence of disease are different in various sweet cherry cultivars, it is important to perform artificial inoculation to secure the infection. Fruit of sweet cherry (*Prunus avium* L.) cultivars: 'Burlat' (Bigarreau Burlat, syn. Bigarreau Hatif), 'Priusadebnaja', 'Asenova