

# Genus *Monilinia* on Pome and Stone Fruit Species

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## SUMMARY

Different species of the genus *Monilinia* are common plant pathogens that endanger pome and stone fruit production worldwide. In Serbia, two species of this genus are widely distributed – *M. laxa* and *M. fructigena*, while *M. fructicola*, which is officially on the A2 EPPO List of quarantine pest organisms in Europe and on the 1A part I List of quarantine pest organisms in Serbia, has so far been detected only on stored apple and nectarine fruits. The most important control measures against these pathogens include chemical control in combination with adequate cultural practices, particularly under favourable conditions for disease development. Concerning that species of this genus can cause significant economic losses, knowledge of the pathogen biology, disease epidemiology and pathogen-host interactions is a necessary prerequisite for stable and profitable production of pome and stone fruits.

**Keywords:** Symptoms; Disease cycle; Identification; Control

## INTRODUCTION

Pathogenic fungi belonging to the genus *Monilinia* are common plant pathogens with a worldwide distribution. In economic terms, they are one of the most important limiting factors for fruit production all over the world. Damages and losses occur primarily on crops of the family *Rosaceae* on which they cause various symptoms – blossom, twig and branch blight, as well as fruit rot. The host range of *Monilinia* spp. includes apple, pear, quince, sweet cherry, sour cherry, apricot, plum, peach, nectarine, almond, etc. (Byrde and Willets, 1977).

Three species of *Monilinia* spp. are considered to be economically significant: *M. fructigena* (Aderhold and Ruhland) Honey, *M. laxa* (Aderhold and Ruhland) Honey and *M. fructicola* (Winter) Honey (Ogawa et al., 1995). *M. fructigena* is the most thoroughly studied species in Europe, especially on apple and pear, (Jones and Aldwinckle, 1990), causing one of the most important diseases on pome fruits (Hrustić et al., 2012a). It primarily causes fruit rot, before and after storage and marketing. On the other hand, *M. laxa* is economically more important on stone fruits, causing mainly blossom and twig blight (Byrde and Willets, 1977),

although it has also been detected on pome fruits (Muñoz et al., 2008). *M. fructicola* is mainly a blossom, twig and fruit pathogen of stone fruits (Fulton, 1999).

Under favourable weather conditions, the disease caused by these pathogens develops rapidly. Heavy rains in the period of blooming, temperatures ranging from 20 to 25°C during the day and cold nights are ideal conditions for pathogen spreading.

Two species of this genus, *M. laxa* and *M. fructigena*, are widely distributed in Serbia. They regularly appear

on pome and stone fruits every year, causing particularly high yield losses when rainy period coincides with blooming and fruit ripening (Hrustić et al., 2010, 2012). As a consequence of blossom blight and fruit rot, losses in stone fruit production can reach 100% (Balaž, 2000), while 5-25% of infected fruits become additionally devastated after harvest (Ivanović and Ivanović, 2001). *M. fructigena* has been found in apple storages with a share of 14.4% compared to the other pathogens (Hrustić et al., 2012a).



Figure 1. *Monilinia laxa*: blossom blight on sour cherry



Figure 2. *Monilinia laxa*: plum (left) and sweet cherry (right) fruit rot, natural infection

## *Monilinia laxa*

**Taxonomic group:** Fungi, Ascomycota, Helotiales, Sclerotiniaceae

**Teleomorph:** *Monilinia laxa* (Aderhold and Ruhland) Honey

**Anamorph:** *Monilia laxa* (Ehrenb.) Sacc. & Vogliano

*M. laxa* is economically one of the most important causal agents of blossom, twig and branch blight of stone fruits (Holb, 2006). It is present in all fruit production regions in the world (Balaž, 2000). Blossom blight is a characteristic symptom which occurs on a range of host plants, such as sweet cherry, plum, peach, nectarine, apricot and almond, as well as on some pome fruits (Holb, 2008). Besides losses caused during the cropping season, significant damages also occur on fruits, particularly during storage (Holb, 2006).

Among stone fruit species, apricot is the most susceptible to blossom blight, followed by sweet cherry, peach, sour cherry and plum (Holb, 2008). On the other hand, damages caused by the pathogen on sweet cherries are more significant on fruits than on twigs and blossoms (Holb, 2006).



**Figure 3.** *Monilinia laxa*: mummified plum fruits

As a consequence of blossom blight and fruit rot, heavy damages occur in orchards (up to 50%), while 5–25% of infected fruits rot away during transport (Ivanović and Ivanović, 2001). In Serbia, the highest losses were observed in the Vojvodina province in 1999 when damages on sour cherry caused by blossom and twig blight reached 100% (Balaž, 2000). Significant losses have also been recorded in other countries: in California for example losses have exceeded 30% during storage (Ogawa and English, 1991; Hong et al., 1997); nectarine yield was completely devastated in the early 1950s (Ogawa et al., 1995); losses in storage reaching 59% in certain years have been reported in Spain (Larena et al., 2005). It is believed that losses during storage and transport are much more serious than those occurring during the growing season (Ogawa and English, 1991).

## Symptoms

Symptoms caused by *M. laxa* are visible on blossoms, branches, twigs and fruits as blossom, branch and twig blight or fruit rot. Various pathological changes caused by *M. laxa* on different plant organs are described by the different names for the disease: brown blossom blight, twig blight, brown canker rot, brown fruit rot or European brown rot (Anonymous, 1997a). The most commonly used name for the disease in literature is brown fruit rot and twig and blossom blight of stone fruits (Ivanović and Ivanović, 2001).

First disease symptoms in the form of blossom blight occur in the spring (Holb, 2008). Macroconidia or ascospores infect blossoms early in the season, during blossom period, usually through stigma and stile of the pistil. Critical phases of blossom infection are from bud formation to petal fall. Studies show that blossoms are the most susceptible in the phase of full bloom (Holb, 2008). All parts of the blossom can be infected, and the symptoms manifest as stamen blight, pistil, sepal and coronal slices necrosis. After penetration in plant tissue, the fungus sporulates very quickly and covers the infected tissue with masses of grayish conidia (Balaž, 2000). Diseased tissue becomes light brown and the whole blossom becomes necrotic. This type of symptoms (blossom blight) is particularly common on sour cherry and apricot.

Although infected flowers can fall off, they mostly remain attached to branches for a substantial period of time, enabling the pathogen mycelium to penetrate through peduncles into twigs and branches,

where it continues development causing oval, elliptic, sunken brown spots and canker lesions, twig and leaf blight (Holb, 2008). Besides indirect penetration of the pathogen through blighted blossoms or stalks of rotting fruits, the infection can also spread directly through wounded bark. In humid conditions, resin extrudes from spots formed on branches, and sporulation occurs on the bark surface (Holb, 2008). Under conditions existing in Serbia, especially on apricot, peach and sour cherry, twigs are usually annularly infected and the part above the infection site decays (Ivanović and Ivanović, 2001). Wilting and blighting of upper twig parts occurs 7-12 days after blossom blight. From thin twigs, the parasite can spread onto thicker branches, i.e. older parts of the tree.

The fruits of pome and stone fruit species can be infected in all developmental phases, from fruit setting to full maturity, as well as during transport, storage and marketing. However, unripe fruits are much less susceptible than ripening ones. The highest damage occurs when fully formed fruits get infected just before harvest. The parasite penetrates into fruits through wounds on the fruit epidermis but infection can also occur at the point of contact between healthy and diseased fruits. First symptoms can be seen as small, circular, brown spots formed as halo around the infection site, mostly wounds. These injuries are usually caused by insects which damage fruits and enable penetration of the pathogen. With the disease development, the spot spreads and brown rot completely invades the fruit within several days under conditions of high temperature and humidity. Brown spots are smooth at the beginning, yet the parasite soon sporulates within the spot and small ruptures appear on fruit epidermis through which whitish at first and then greyish pillow-like sporodochia extrude, usually as concentric rings. When brown rot completely invades the fruit, it begins to wilt, dry and shrivel. Mycelium of the fungus completely permeates fruit tissue, transforming it into a stroma, which does not disintegrate but hardens and becomes a mummified structure hanging on a branch, or falling on the ground. Fruit brown rot under conditions of high air humidity completely invades sweet and sour cherry fruits after 4-5 days, and apricot, plum and peach fruits after 8-10 days (Holb, 2008).

### Disease cycle

*M. laxa* overwinters as a mycelium in canker lesions in the bark of diseased twigs and branches, blighted blossom parts and mummified fruits in the canopy or

on the ground. Under humid conditions, mycelium sporulates on these plant parts, conidia are formed during winter and spring and the infection spreads further (Byrede and Willets, 1977; Holb, 2008). Conidia are formed under humid conditions in piles – sporodochia. Conidia formed in the spring on blighted blossoms and branches, as well as conidia on mummified fruits, are a source of inoculum for ripening fruits (Ogawa et al., 1995). On mummified fruits, the largest number of conidia is formed just before blossom-time, while the largest number of conidia on blossoms infected during springtime is formed in the first two months after the establishment of infection. On newly infected blossoms, a ten times higher number of conidia can form, compared to overwintered ones. However, the number of conidia formed on overwintered fruit mummies is more than 10 times higher than on newly infected flowers (Holb, 2008). Conidia formed under humid conditions in the spring are spread by wind, rain drops and insects (Byrede and Willets, 1977; Holb, 2008). During the season, pathogen has several cycles of secondary infections (Holb, 2008).

Under conditions of warm and dry weather, the critical phase of infection is very short and possibility for infection establishment is low. A long blooming period caused by low temperatures and frequent rainfalls is an ideal condition for high disease incidence (Trkulja, 1996). Optimal temperature for disease development is 24°C, although infection and symptom development occur in a very wide temperature range (4-30°C) (Holb, 2008). When conidia reach susceptible tissue, they germinate within 2-4 h and, under conditions of favourable wetness duration and temperature, first signs of necrosis can be observed within 3-6 days (Holb, 2008).

### *Monilinia fructigena*

**Taxonomic group:** Ascomycetes, Helotiales, Sclerotiniaceae

**Teleomorph:** *Monilinia fructigena* (Aderh. & Ruhland) Honey ex Whetzel

**Anamorph:** *Monilia fructigena* (Pers. ex Pers.) Eaton  
*M. fructigena* is the best known and economically the most significant species of the genus *Monilinia* on apple and pear in Europe (Jones and Aldwinckle, 1990). It primarily causes fruit rot, before or during storage, while blossom, twig and branch infections are rare (Anonymous, 2004). Although the most significant yield losses are on pome fruits,



*M. fructigena* can also infect stone fruits, among which plum is the most susceptible (Byrde and Willetts, 1977; Anonymous, 2004). In Serbia, it has also been detected on some grape cultivars (Stojanović and Kostić, 1958).

*M. fructigena* is present throughout Europe, Asia (Near and Far East, India), North Africa, and some parts of South America (Batra, 1991).

## Symptoms

Fruit rot is the primary and most common symptom of the disease (Jones and Aldwinckle, 1990; Holb, 2006), while symptoms rarely occur on blossoms and twigs (Anonymous, 2004). Fruits can be infected from fruit setting to harvest, but the highest damage occurs if the infection is established during late ripening. Disease incidence on fruits can be high when they are injured by insects or hail. Similarly, significant losses occur on stored apple and pear fruits.

In the initial phase of disease development, circular, concentric light brown spots are formed on fruits. The spots rapidly spread, the tissue within spots becomes rotten and pathogen invasion of the whole fruit follows, and characteristic structures form on the fruits – circular sporodochia (Holb, 2008). This type of rot is known as brown rot. *M. fructigena* also causes black rot which occurs on fruits stored in the dark. The rotten and infected parts of the fruit have dark or black colour on which no sporulation is present (Balaž, 2000; Ivanović and Ivanović, 2001).

In both cases, mycelium of the fungi completely permeates the fruits which shrivel due to water loss, and turn into stromatic structure. Dried fruits hang in the canopy attached to twigs and branches or fall off. Depending on temperature, the whole fruit becomes rotten within 7–14 days.

Besides symptoms on fruits, infections of all parts of the flower are possible in the spring, while twig blight and canker lesions are rarely observed as an extension of blossom infection (Anonymous, 2008).

## Disease cycle

*M. fructigena* usually overwinters in mummified fruits in tree canopies or on the ground but also in infected branches and in canker lesions. Under favourable humidity in the following spring or summer, reproductive structures of the pathogen – sporodochia,

form, on the surface of infected parts. Conidia are wind-, rain- or insect-borne to the fruits. High temperatures and humid conditions are favourable for conidia germination and infection establishment. Fruits can be infected by direct penetration of germination tube through the fruit cuticle, stomata or trichomes, but also through wounds and injuries caused by insects or unfavourable environmental conditions. The infection usually occurs at the site of injury, but it can also develop on the point of contact of infected and healthy fruits (Anonymous, 2008).

*M. fructigena* rarely forms apothecia (Batra and Harada, 1986). Many authors have tried to prove their existence in natural conditions for years. The first finding of apothecia was registered 100 years ago (Aderhold and Ruhland, 1905, cited in Batra and Harada, 1986) and later it was confirmed by apothecia found on overwintered apple fruits partially covered by soil. Several successful attempts to provoke apothecia formation in the laboratory have been published (Batra and Harada, 1986), which undoubtedly proved the possibility of their formation.

*M. fructigena* has several cycles of secondary infections during vegetation, which are initialized by conidia formed on initially infected fruits. Incubation period lasts about 6–7 days (Ivanović and Ivanović, 2001). *M. fructigena* is a pathogen favouring high relative air humidity and temperatures above 5°C. Light stimulates sporulation and partially inhibits conidia germination (Anonymous, 2008).

## *Monilinia fructicola*

**Taxonomic group:** Fungi, Ascomycota, Helotiales, Sclerotiniaceae

**Teleomorph:** *Monilinia fructicola* (Winter) Honey

**Anamorph:** *Monilia fructicola* L.R. Batra

*M. fructicola* causes severe damage, particularly to stone fruit species, during vegetation as well as during storage. It parasitizes plant species from the sub-family Prunoideae more often than Pomoideae (Holb, 2008), and it is mainly present on peaches and nectarines (Wilson and Ogawa, 1979).

The pathogen has been detected in Australia, New Zealand, South Africa, North and South America and Japan (Anonymous, 1997), while in Europe, it is on the A2 EPP0 List of quarantine organisms (Anonymous, 2009). However, in recent years it has been detected in several European countries: France (in 2001), Spain (2005), Hungary (2007), Italy (2008), Germany (2009), Slovenia (2009), Switzerland

(2009), Poland (2010), Romania (2010) and Serbia (2012) (Baker et al., 2011; Vasić et al., 2012; Hrustić et al., 2012b).

The spread of this pathogen can have very serious consequences, especially when temperatures favourable for pathogen development occur during fruit ripening phase and rainy harvesting period. *M. fructicola* causes the most severe damage after harvest, during storage and transport (Ogawa and English, 1991; Hong et al., 1997). The highest losses caused by this pathogen have been recorded in North America on peaches, sweet cherries and plums. It causes significant losses during fruit storage in California, frequently over 30% (Ogawa and English, 1991; Hong et al., 1997), or even up to 80-90% (Hong et al., 1997; Larena et al., 2005).

## Symptoms

The pathogen can infect all above-ground plant organs: blossoms, buds, branches, twigs and fruits (Ogawa et al., 1995). Leaf infection has also been found on plum, (Michailides et al., 2007). However, *M. fructicola* mostly infects the fruits, which discriminates this pathogen from *M. laxa* which usually infects blossoms and branches/twigs (Ogawa et al., 1975). Symptoms caused by *M. fructicola* are very similar to symptoms caused by *M. laxa*, an important difference being that *M. fructicola* mycelium can remain concealed on just set, very young fruits until the beginning of fruit ripening when the pathogen becomes visible (Batra, 1991; Emery et al., 2000; Gell et al., 2008).

*M. fructicola* has two phases in disease development: the phase of blossom blight and the phase of fruit rot (Luo et al., 2005). Under favourable environmental conditions, early in the spring, spores (conidia and ascospores) infect blossoms and also immature fruits (Gell et al., 2009). Blossoms are most susceptible at full bloom (Michailides et al., 2007). Under unfavourable environmental conditions, primary infections may remain latent in blossoms and/or immature fruits (Emery et al., 2000; Gell et al., 2008) during the whole cropping season, until weather conditions become optimal for disease development (Gell et al., 2008; Luo et al., 2001; Luo and Michailides, 2003). If favourable weather conditions occur, the pathogen can progress and reach branches and twigs from blossoms, causing twig blight and canker lesions on branches. Secondary inoculum, which can cause latent infections on immature fruits, as well as brown rot on ripe stone fruits, is formed on diseased fruit parts (Holtz et al., 1998).

Optimum temperature for blossom infection ranges from 20 to 25°C, while temperatures below 10°C and above 30°C are unfavourable for infection establishment (Luo et al., 2001). Besides optimum temperature, wetness duration for at least 3-4 h is necessary for infection establishment (Luo and Michailides, 2001a).

*M. fructicola* infects fruits through injuries or natural openings on fruits (Michailides and Morgan, 1997), only when all conditions necessary for infection are present: favourable weather conditions, a host in susceptible phenophase and a virulent pathogen (Luo et al., 2001). Fruits can be infected in all developmental phases, yet they are more susceptible in the ripening phase (Ogawa et al., 1995). Luo and Michailides (2001) found that young, immature plum fruits are more susceptible to the disease than fruits in other developmental phases. Similarly, Biggs and Northover (1988) proved that young peach fruits are very susceptible to pathogen penetration, after which they become resistant until 2-3 weeks before the full ripening when fruit susceptibility to the disease rapidly increases. Infected fruits rot away within a few days, remain attached to branches or fall to the ground. On these fruits, stroma which are necessary for apothecia formation, are formed (Holtz et al., 1998). Fruits then dry out, turn into mummies – a characteristic indicator of the disease (Byrde and Willetts, 1977).

## Disease cycle

The pathogen can overwinter in the form of mycelium: in mummified fruits attached to the tree, on which conidia are formed during the spring; in mummified fruits on the ground surface, on which apothecia are formed; or in blossom parts, twigs and canker lesions (Holb, 2008).

The primary development cycle of the disease starts with conidia formed on the mummified fruits or other infected plant parts (Holb, 2008). An additional source of inoculum can be pseudosclerotia formed on the mummified fruits on which apothecia with ascospores are formed. In humid conditions ascospores infect blossoms (Byrde and Willetts, 1977). Conidia are spread by rain, wind and insects.

Under favourable environmental conditions, ascospores and conidia formed on mummies cause blossom blight in the spring (Holb, 2008). After blossom infection is established, initial hyphae infect blossom parts until they are completely colonized. The mycelium penetrates through blossom parts to blossom

stalks and twigs. Infected blossoms are the source of secondary inoculum for fruit infection (Holb, 2008).

High relative air humidity and mild temperatures are necessary for conidia to establish the infection. Conidia of *M. fructicola* can germinate in a wide temperature range (0–35°C). Germination decreases from 0°C to 5°C, and is completely inhibited at temperatures above 38°C (Casals et al., 2010). Optimum temperature for conidia germination is between 15°C and 30°C. Depending on environmental conditions, a new generation of conidia is formed seven days after infection, and several generations of conidia can be formed during vegetation.

The teleomorph stage, rarely formed by the European species *M. fructigena* and *M. laxa*, is important in the life cycle of *M. fructicola*. The teleomorph stage of *M. fructicola* Batra was first detected and described on overwintering mummified apple fruits (Batra and Harada, 1986). No formation of apothecia has been recorded for *M. fructicola* in Europe (Villarino et al., 2010), but it has been detected on other continents (Holtz et al., 1998).

### Identification of *Monilinia* species

Identification of species of the genus *Monilinia* can be difficult (Anonymous, 2008), because they are hard to discriminate based on morphological properties. Symptoms caused by these species are characteristic and easily recognised. However, it is hard to determine precisely which species of the *Monilinia* genus is the causal agent (Michailides et al., 2007). *M. laxa* causes more significant damage on blossom and twigs, while *M. fructicola* is much more destructive on fruits (Ogawa et al., 1975). *M. laxa* is more common on almond and apricot, and *M. fructicola* on peach, nectarine and plum fruits (Michailides et al., 2007). *M. fructigena* usually causes symptoms on pome fruits, while *M. laxa* and *M. fructicola* are frequently found on stone fruits.

Standard methods for precise identification of *Monilinia* species include: investigation of morphological and cultural features (Byrde and Willetts, 1977; De Cal and Melgarejo, 1999) and/or molecular methods (Hughes et al., 2000; Côté et al., 2004).

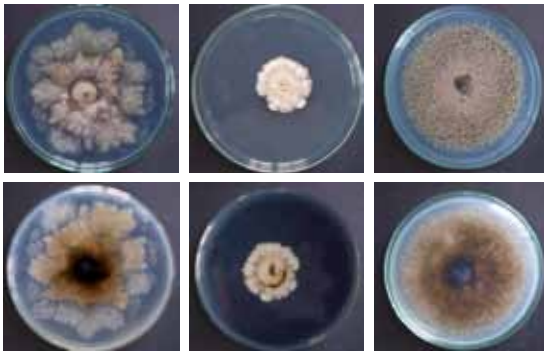
Identification based on morphological features of isolates is time consuming and requires great experience, and on the other hand, can lead to false conclusions (van Leeuwen and van Kesteren, 1998; De Cal and Melgarejo, 1999). Misidentification is a consequence of morphological differences commonly

present among isolated colonies of the same species (van Leeuwen and van Kesteren, 1998). According to Lane (2002) there is no specific morphological character which would reliably discriminate species of this genus, yet the three species of the genus can be discriminated by investigating several morphological characteristics simultaneously (Lane, 2002). The synoptic key for discrimination of morphologically similar species of the genus *Monilinia* Lane (2002) specifies the following characters: colony colour, growth rate, sporulation, concentric ring of spores, colony margins, lobation, and black zones – margins.

*M. fructicola* differs from *M. laxa* by an entire colony margin, colour of colony and longer germination tube before branching (Anonymous, 2009). *M. fructicola* sporulates abundantly and conidia produced are somewhat smaller than the conidia of *M. fructigena* (Anonymous, 2009). *M. fructigena* also has the whole colony margin, different colour of colony and longer germination tube than *M. laxa* (Anonymous, 2009). An important discrimination character is length of germination tube and branching mode. On water agar, conidia of *M. laxa* form short germination tubes (their length from conidia to the first branching site is less than 60 µm). On the other hand, *M. fructigena* and *M. fructicola* form germination tubes over 220 µm long before branching. Besides, *M. fructigena* and *M. fructicola* can be clearly discriminated based on colony growth rate under UV/dark light regime. Under these conditions, *M. fructigena* reaches maximum growth rate of 8 mm, while maximum growth rate of *M. fructicola* is 20 mm/day (De Cal and Melgarejo, 1999). Batra (1979) has also found that *M. laxa* can be discriminated from *M. fructigena* and *M. fructicola* by slower growth rate: *M. fructigena* grows 11 mm/day, while *M. laxa* grows about 7 mm/day. Therefore, mycelium growth rate on potato-dextrose agar medium (PDA) can be a useful discrimination criteria for *Monilinia* species.

On the other hand, Batra and Harada (1986) detected the formation of black, irregularly shaped sclerotia in *M. fructigena* cultures and regarded this character as specific for *M. fructigena* isolates, which is in agreement with our previous investigations (Hrustić et al., 2012) – cultures of *M. fructigena* derived from quince fruits formed sclerotia after 14 days of incubation.

Although these characteristics can be useful in identification of typical colonies of *Monilinia* genus, atypical cultures of *M. laxa* can easily be misidentified as *M. fructigena* or *M. fructicola* (De Cal and Melgarejo, 1999).



**Figure 4.** *Monilinia laxa* (left), *M. fructigena* (middle) and *M. fructicola* (right) colonies, grown on potato dextrose agar for 10 days: a) upper surface; b) lower surface

For fast and reliable identification of isolates, the utilization of molecular methods, primarily PCR technique, is necessary (Côté et al., 2004). Detection of *Monilinia* species using primers and protocols described by Côté et al. (2004), is a useful, fast and reliable technique that ensures precise identification of isolates in only one reaction. Using the specific primers MO368-5, MO368-8R, MO368-10R and Laxa-R2, amplicons of different predicted sizes are obtained: 402bp specific for *M. fructigena*, 535bp for *M. fructicola*, 351bp for *M. laxa* and 425bp for *M. polystroma*.

## CONTROL OF MONILINIA SPECIES

Adequate protection measures against plant pathogens as a necessary precondition for stable and profitable agricultural production are largely dependent on the level of knowledge about pathogen biology, disease epidemiology and parasite-host relationship. Only a wide knowledge of all factors directly or indirectly effecting disease development can ensure the application of a complex of measures that will prevent losses (Tanović et al., 2011).

Control of pathogenic fungi of the *Monilinia* genus, the causal agents of diseases on many fruit species, is very demanding and oriented in several directions:

- Breeding of resistant varieties;
- Cultural practices;
- Biological measures;
- Chemical control.

### Breeding of resistant varieties

Breeding of resistant varieties, as one of the simplest and the most effective ways for *Monilinia* spp. management does not get enough attention in Serbia at present.

Consequently, available literature data are also insufficient. Jordović (1954) investigated fruit susceptibility of ten leading peach varieties to *Monilinia* spp. and found that none exhibited full resistance, while the observed differences between some varieties were insignificant. Also, the author pointed out that peach fruit susceptibility to *Monilinia* spp. increased as the ripening progresses, and that fruit resistance or susceptibility heavily depends on the firmness and colour of fruit flesh, firmness and thickness of epidermis, epidermis hair overgrowth and other pomological properties of the fruit. According to Jevremović (1976), peach varieties with the greatest vigour and lower yield are less susceptible, while those with less vigor and high yielding are more susceptible to *Monilinia* spp.

Worldwide experience has shown that sweet cherry varieties with thinner epidermis are much more prone to penetration of these pathogens than varieties with thicker epidermis. The varieties Bigarreau Burlat, János cseresznye and Valerij Cskalov are highly resistant to blossom blight (Holb, 2006). Concerning sour cherry, the varieties Lativiszskaja Nizkaja, Nagy Angol, Mocanesti, Ljubszkaja, Sirpotreb, Oblacsinszkaja, Cigánymeggy 3, Maraska Savena, Mettar and Elegija are mildly susceptible to *M. laxa* (Holb, 2006). When it comes to apricot and peach, several apricot varieties grown in Romania (Neptun, Mamaia, Silvana, Sulina and Sirena) are considered tolerant to the causal agents of brown rot (Holb, 2006). The plum varieties čačanska najbolja and President are moderately, and Besztercei, Silvia and Tuleu gras are mildly susceptible to fruit brown rot caused by *M. laxa* (Holb, 2006).

No data is available on efforts to breed varieties resistant to *M. fructicola* (Holb, 2008).

### Cultural practices

Various studies have shown that cultural practices can reduce inoculum and provide microclimatic conditions favourable for plant and unfavourable for the pathogen. Cultural practices, site selection, planting density, growth habit, variety selection, breeding of less susceptible varieties, etc., reduce the risk of disease incidence (Kišpatić and Maceljki, 1989).

When establishing an orchard, inclined terrain should be selected and rows should be oriented in the direction of dominant winds for better ventilation. All hygiene measures should be regularly applied in the orchard (canopy maintenance, weed control, etc.) to increase plant vitality and thus reduce pathogen pressure. When all plants are adequately and equally manured,



they are less susceptible to attacks by *Monilinia* species (Trkulja, 1996). Elmer et al. (2007) found that calcium content after manure increased for minimum 50% and that fruits containing more calcium in the epidermis were much less prone to infection. If calcium treatment is done before harvest, fruit brown rot incidence is significantly reduced during storage. Also, plants should be sufficiently and regularly watered in the ripening phase because long droughts followed by heavy and long lasting rain cause the fruits to crack and the parasites easily penetrate the fruits through the injuries (Trkulja, 1996).

Regular harvest, removal of plant residues after harvest, cutting and burning of diseased and blighted twigs, collecting and burning of mummified fruits, avoiding unnecessary plant injuries during regular cultural practices, as well as reduced planting density, can significantly cut down disease incidence. Actually, destruction of primary sources of inoculum is the most important control measure (Leeuwen et al., 2000, 2002). Mummified fruits on the ground as well as on trees should be collected in the autumn or in early spring (Leeuwen et al., 2000, 2002). Also, if possible, diseased fruits should be removed during the summer after first disease symptoms have been observed. These measures can be very useful in small orchards or yards because they significantly reduce infection potential of the parasite. However, such measures are difficult to apply on larger plantations, where deep tillage of mummified fruits is a more useful option.

Investigations by Borve and Stensvand (2003) showed a good efficacy of covering sweet cherry trees during the rainy period from blooming to harvest. They showed that rain covers can serve as an addition to or even substitute for fungicide treatments.

### Biological measures

Biological control is a unique way of crop protection against pathogens based on the employment of microorganisms instead of conventional fungicides, or as their supplement in order to reduce the quantity of chemical substances used in agriculture. Most reports on the use of fungi, bacteria and yeasts have been made in the past twenty years. Several biological control agents (BCA) have been successfully developed, and some of them are already registered for use in fruit production.

*Bacillus subtilis*, a bacterium long known for its antimicrobial activity, is an active ingredient of several products registered worldwide (Tomlin, 2009). Pusey et al. (1984) pointed to a possibility of controlling

*Monilinia* species by *B. subtilis*. On the other hand, investigations conducted by Tanović et al. (2005, 2010a) have confirmed high efficacy of biofungicides based on *B. subtilis* under laboratory conditions but less than satisfactory efficacy in the field against *Botrytis cinerea* in raspberry. In Croatia, the yeast species *Aureobasidium pullulans* (strains DSM 14940 and 14941) has been registered for control of *M. fructigena* in apple, pear and quince (Lučić, 2009), while Wittig et al. (1997) reported a significant reduction in infection potential of *M. laxa* after applying the same yeast at the blooming phase on sweet cherry. The use of a formulated product based on *Trichoderma harizanum* in combination with fungicides has given good results (Elad, 1994). Satisfying results have been achieved by a product based on oospores of *Pythium oligandrum* (Filajdić et al., 2003; Miletić et al., 2003). De Cal et al. (2009) pointed to a possibility of using *Epicoccum nigrum*, a BCA, in management of *Monilinia* spp. conidia found on fruit surface. Melgarejo et al. (1985) found that five species (*Aspergillus flavus*, *E. nigrum*, *Penicillium chrysogenum*, *P. frequentans* and *P. purpurogenum*) inhibit hyphal growth and conidia germination of *M. laxa*. De Cal et al. (1988) recorded that *P. frequentans* produces substances with antifungal activity against *M. laxa*. Madrigal and Melgarejo (1994) went one step further in their investigations of the mode of action of biological agents and found out that *E. nigrum* produces flavipin, a substance that inhibits respiration and in that way inhibits conidia germination of *M. laxa*. It was also found that lytic enzymes produced by *P. purpurogenum* stimulate degradation of hyphae and spores of *M. laxa* (Larena and Melgarejo, 1996). Chand-Goyal et al. (1996) pointed out good results in prevention of *Monilinia* spp. by combined application of *Cryptococcus laurentii* and *C. infirmo-miniatus* with small quantities of iprodion.

Besides, medicinal and aromatic plants are known to be an important source of substances with antimicrobial activity. Previous investigations (Tanović et al., 2009, 2010; Hrustić et al., 2011) showed that the volatile phase of some essential oils is highly toxic to post-harvest fruit pathogens. In an investigation which included essential oils from more than 30 plant species, it was found that the volatile phase of thyme oil has the strongest inhibitory effect. In *in vitro* and *in vivo* experiments conducted by Hrustić et al. (2011), strong antimicrobial activity of thyme oil against *M. fructigena* was confirmed. On the other hand, researches by Grhovac et al. (2012) showed that out of 56 tested oils, 47 had no fungicidal effect on *Colletotrichum* spp. even

at concentration of 0.16 µl/ml of air, and that oregano and thyme oils were the most effective against the causal agents of apple bitter fruit rot.

However, production and use of biopesticides, although a desirable solution from the standpoint of food safety, is still too costly to be economically acceptable, compared to the significantly lower costs of conventional fungicides (Elad, 1994).

## Chemical control

As all these listed measures, although useful, are not efficient enough for successful protection of different fruit species from *Monilinia* spp., and that economic losses can be severe, regular chemical control is necessary, particularly in years with favourable conditions for disease development.

In Serbia, about 10 active ingredients and their combinations have been registered for control of *Monilinia* spp. (copper oxychloride, prochloraz, cyprodinil + fludioxonil, prosimidon, thiophanat-methyl, vinclozolin, carbendazim, iprodion, tebuconazole, boscalid + pyraclostrobin, difenoconazole, propiconazole, chlorothalonil) (Anonymous, 2011), while over 30 active ingredients from different chemical groups are being used worldwide for the same purpose (azoxystrobin, bitertanol, captan, dichlofluanid, dithianon, fenhexamid, fludioxonil, fluopyram, fluoroimid, imibenconazole, mepanipyrim, oxiconazole fumarate, propineb, triadimephon, triflumizol, tiram, triforin and ciram) (Tomlin, 2009).

Fungicides were for the first time used against *Monilinia* spp. when copper fungicides were introduced in the early and mid-1920s (Holb, 2006). Bordeaux mixture and similar substances were applied for winter treatments, which significantly reduced the infection potential of overwintering pathogen (Holb, 2005). Today, fruit protection protocol against *Monilinia* species also starts with preventive winter treatments using copper based fungicides.

Control strategy for *Monilinia* species, depending on host plant, variety and environmental conditions, relies on fungicide applications during the blooming period. Since blooming is a critical phase of infection, control includes 2-3 fungicide treatments from the phenophase 59 to 67 (Meier, 1997), and 1-2 treatments during fruit ripening before harvest (Rüegg et al., 1997; Zehr et al., 1999). Spiegel and Stammer (2006) take the standpoint that during development and ripening of fruits up to five treatments are needed, while Yoshimuri et al. (2004) suggest 1-2 fungicide treatments per year in plum, sweet and sour cherry plantations, and 3-4 treatments for peaches and nectarines.

For control of *Monilinia* species, fungicides that affect sporulation are the most commonly used: dicarboximides, benzimidazoles and triazoles. In addition, protective fungicides with nonspecific modes of action (captan, mancozeb, metiram, propineb, tiram, folpet, chlorotalonyl and ciram) are also frequently used (Baker et al., 2011). Investigations have shown that fungicides including: benomyl, thiophanat-methyl, vinclozolin, iprodion, bitertanol and triforin are highly effective in controlling *Monilinia* species (Harman and Beever, 1987; Takamura and Ochiai, 1989). Until the 1980s, the most important fungicides used for control of *Monilinia* species had been benzimidazoles, and among them benomyl and thiophanat-methyl were mostly used (Ma et al., 2003). In the European Union, benomyl which had been used in *Monilinia* spp. control for many years, has been excluded from Annex 1 Directive 91/414/EEC (now 1107/2009) (EU Pesticide Database, 2012) because of its adverse toxicological properties (reproduction toxicity and mutagenicity) and therefore is no longer in use for fruit protection. Also, benomyl was successfully used for the control of blossom blight in California from its registration in 1972 until 1977 when resistance to this substance was recorded (Sonoda et al., 1983; Michailides et al., 1987). Since 1977 when resistance of *Monilinia* spp. to benzimidazoles was first detected, ergosterol inhibitors (DMI fungicides) have become the most efficient and most widely used fungicides against species of this genus (Zehr et al., 1999; Schnabel et al., 2004). They are very efficient in prevention of blossom blight and fruit rot on stone fruit species (Schnabel et al., 2004). Propiconazole, tebuconazole and fenbuconazole have become a standard for chemical control of these species in peach plantations in southeastern parts of the USA. Despite successful use of these fungicides for over 15 years, reduced sensitivity of isolates has been recently detected in several regions in Eastern USA (Zehr et al., 1999; Schnabel et al., 2004).

In recent years, new groups of fungicides with different modes of action have been registered: strobilurines, carboxamides, hydroxianilides and anilino pyrimidines, which are proposed for use against the causal agents of brown rot. However, strobilurine, azoxystrobin and pyraclostrobin fungicides are considered to be high risk fungicides in terms of resistance development (Amiri et al., 2008). Actually, reduced sensitivity of the pathogens to these groups of substances has been registered in a number of apple orchards and vineyards (Gullino et al., 2004; Köller et al., 2004), but not for fruit rot causal agents on peaches (Amiri et al., 2008). Other examples of new products with different modes of action are

fenhexamid and boscalid. Fenhexamid is hydroxianilide which strongly inhibits primary hyphae elongation and mycelial growth at very low concentrations (Tanović et al., 2011), while boscalid from the carboxamide group, as well as azoxystrobin and pyraclostrobin, inhibit respiration and are mainly used as protective fungicides, although curative and eradicated action was also registered (Wong and Wilcox, 2002). Fenhexamid has very narrow activity spectrum – it is efficient against *Monilinia* spp. and related species (*Sclerotinia* spp. and *Botrytis* spp.) (Tanović et al., 2011). Azoxystrobin and boscalid + pyraclostrobin have proved to be as efficient as DMI fungicides. Other researches show that boscalid + pyraclostrobin can be even more efficient than DMI fungicides (Ritchie and Pollard, 2002) against blossom blight and fruit rot on stone as well as on pome fruits (Spiegel and Stammler, 2006).

In contrast to *M. laxa* and *M. fructicola*, *M. fructigena* causes significant losses only when fruits are damaged. Therefore, the most important measure against *M. fructigena* is control of insects that injure fruits and open entryways for this pathogen (Holb, 2006). Special chemical treatments are not usually recommended for *M. fructigena* control on pome fruits if regular chemical protection from other diseases and pests is conducted, but 1-2 pre harvest treatments are proposed for stone fruit species (Balaž, 2000). Fungicides registered in Serbia for fruit rot control include trifloxystrobin, trifloxystrobin + captan, carbendazim, cyprodinil + fludioxonil, thiophanate-methyl, boscalid + pyraclostrobin and the bacterium *Bacillus subtilis* (Grahovac et al., 2011).

Application of fungicides in storage is not a regular practice (Mari et al., 2007), however several fungicides for fruit dipping prior to storage have been registered in some countries (Karabulut et al., 2010), such as fludioxonil, fenhexamid, tebuconazole, propiconazole and pyrimethanil in the USA (Vico and Jurick, 2012). Treatment can reduce the inoculum present on fruit surface (Karabulut et al., 2010) but cannot affect the mycelium present inside fruits. On the other hand, the European Union, as well as Turkey, do not allow the use of fungicides after harvest or in storage facilities (Karabulut and Baykal, 2004).

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## Vrste roda *Monilinia* na jabučastim i koštičavim vrstama voćaka

### REZIME

Različite vrste roda *Monilinia* dobro su poznati biljni patogeni koji ugrožavaju proizvodnju jabučastog i koštičavog voća širom sveta. U našoj zemlji široko su rasprostranjene dve vrste ovog roda – *M. laxa* i *M. fructigena*, dok je *M. fructicola* koja se zvanično nalazi na A2 EPPO listi karantinskih štetnih organizama u Evropi i na A1 listi karantinskih štetnih organizama u našoj zemlji, do sada detektovana samo na uskladištenim plodovima jabuke i plodovima nektarine. Najznačajnija mera borbe protiv ovih patogena je hemijsko suzbijanje uz primenu adekvatnih agrotehničkih mera, naročito u godinama povoljnim za razvoj oboljenja. S obzirom da vrste ovog roda mogu prouzrokovati velike ekonomske gubitke, stepen poznavanja biologije patogena, epidemiologije bolesti i odnosa parazit-domaćin predstavljaju neophodan preduslov stabilne i profitabilne proizvodnje jabučastog i koštičavog voća.

**Ključne reči:** Simptomi; ciklus razvoja; identifikacija; suzbijanje