Potential Impact and Management of *Monilinia fructicola* in an Integrated Peach Orchard

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Summary

To assess the potential impact and control options for *Monilinia fructicola*, the alien stone fruit pathogen in Croatia, development of pre-harvest and post-harvest brown rot was monitored during 2014 and 2015 in a peach orchard where integrated pest management measures are implemented. Two experimental locations, A (younger part of the orchard with a lower elevation) and B (older part with higher elevation) were established, and conditions for infection were monitored using a forecast model for *M. fructicola* with PinovaSoft® application. In 2014, symptoms of brown rot in the field were recorded only on fruits at location B, with 6.6% incidence. *Monilinia* spp. developed on 92.6% of stored fruits from location A and on 20.3% of stored fruits from location B. Out of 40 isolates collected from the fruits at the moment of harvest, 27% were *M. fructicola*, 30% were *Monilinia laxa*, and 43% were *Monilinia fructigena*. Similar percentages of three *Monilinia* species were determined on stored fruits. In 2015, no fruits affected with brown rot were found at the moment of harvest, but only after 21 days of storage and only on fruits from location B, with low incidence (8% fruits). *M. fructicola* was found on all fruits with brown rot symptoms, while *M. laxa* and *M. fructigena* were detected only on three fruits in mixed infections with *M. fructicola*. Despite differences in brown rot incidence between years, forecast model recorded seven infections with *M. fructicola* during the vegetation period in 2014 and 15 infections in 2015. The results are indicating that common measures against indigenous *M. laxa* and *M. fructigena* may be suitable for the control of invasive *M. fructicola*, and that all three *Monilinia* species on peach in Croatia can occur in mixed populations.

Key words

brown rot, *Monilinia* spp., disease incidence, population structure

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Introduction

Plant pathogenic fungi *Monilinia laxa* (Aderhold & Ruhland) Honey and *Monilinia fructigena* Honey are among the most important and widespread pathogens on stone fruits in Croatia, causing blossom blight and fruit rot, often called brown rot (Cvjetković, 2010). However, a third *Monilinia* species, *Monilinia fructicola* (G. Winter) Honey, is considered to be the most aggressive *Monilinia* species on stone fruits worldwide (Hong et al., 1998). Until 2014, *M. fructicola* was regulated as a quarantine pathogen within the European Union (EU, 2000), but during the last 15 years it became obvious that this fungus has been established across Europe (Villarino et al., 2010). In 2013, it was recorded for the first time in Croatia (Ivić et al., 2014).

Introduction of *M. fructicola* into Europe has raised questions on potential impact of this alien species to European stone fruit production. Several pest risk analyses have been published (EFSA, 2011; van Leeuwen et al., 2001), with different predictions regarding the possible impact of this pathogen in areas where it has been established. In the risk analysis conducted by the European Food Safety Authority (EFSA), it was concluded that no additional control measures, cultural or chemical, would be needed to control *M. fructicola* in orchards where it has been established. It is presumed that control measures against the indigenous European species *M. laxa* and *M. fructigena* should be sufficient to control *M. fructicola* at the same time (EFSA, 2011). Such prediction is based on the fact that all three *Monilinia* species occurring on stone fruits have similar biology (Byrde and Willetts, 1977). Taking this into account, measures for the control of *M. laxa* and *M. fructigena* on stone fruits, like pruning of infected plant parts, removal of mummified fruits and fungicide applications during the bloom and during the ripening period (Cvjetković, 2010) may be effective in control of co-occurring *M. fructicola*. Contrary to such previsions, some specificities in *M. fructicola* biology, compared to *M. laxa* or *M. fructigena*, could lead to different predictions. For example, van Leeuwen et al. (2001) are stating that *M. fructicola* represents a serious risk for European stone fruit industry, probably causing significant economic losses if established. According to the experiences from other parts of the word, *M. fructicola* can produce apothecia with ascospores as a source of inoculum (Byrde and Willetts, 1977), which is a feature very rarely seen in *M. laxa* or *M. fructigena* (Cvjetković, 2010). Moreover, *M. fructicola* can relatively rapidly develop resistance to fungicides (Chen et al., 2013; May-de Mio et al., 2011; Amiri et al., 2010; Luo and Schnabel, 2008; Schnabel and Dai, 2004).

The impact of an alien plant pathogen in a certain invaded area can objectively be assessed usually only after a relatively long period after its invasion and establishment. Therefore, there are many uncertainties related to the potential impact of *M. fructicola* in Croatia, in orchards where this fungus has established. The main objective of this research is to evaluate the effectiveness of integrated pest management measures in control of *M. fructicola* in a peach orchard, as well as to assess the presence and share of different *Monilinia* species on peach fruits in the field and after harvest.

Materials and methods

Monitoring of *Monilinia* spp. in the field

Development of pre-harvest brown rot (*Monilinia* spp.) was assessed in a peach orchard in Vratišinec (Međimurje County), in North-Eastern Croatia, during 2014 and 2015. In this orchard, *M. fructicola* was found in 2013 (Fazinić et al., 2016) and it was presumed that all three *Monilinia* species are present there. Integrated pest management was implemented in the orchard, and brown rot was controlled using cultural measures (removal of mummified fruits and the eventual blighted shoots) combined with fungicide treatments during the bloom and close to the harvest. The trial was set on two locations within the orchard. The first location was in a younger part of the orchard with a lower elevation (location A), and the second one in an older part with higher elevation (location B). Peach cultivar on both locations was `Redhaven`. The trial was set as the complete randomised block in three replicates. Each replicate included five trees on location A and four trees on location B.

During the vegetation period in both years, conditions for infection were monitored using a forecast model for *M. fructicola* with PinovaSoft® application implemented in agro-meteorological station Pinova Meteo®. The forecast model recorded the conditions for infection with *M. fructicola* and showed the predicted moments of infections.

Disease incidence was assessed three times during the vegetation period in both years. On flowers, the incidence of blossom blight was assessed on 5 April 2014 and on 18 April 2015 according to EPPO (1998). Ten blossoms on five shoots on each tree were assessed for blossom blight symptoms. In July, disease incidence of brown rot on fruits was assessed two times according to EPPO (2002), 10-13 days before the harvest (4 July 2014 and 17 July 2015) and at the moment of harvest (17 July 2014 and 27 July 2015). Fifty fruits per tree were assessed for brown rot symptoms. In 2014, 40 fruits with brown rot symptoms were collected at the moment of harvest and were transferred to the laboratory for the identification of *Monilinia* species. In 2015, no fruits with brown rot symptoms were present at both experimental locations at the moment of harvest and no symptomatic fruits were collected.

Monitoring of *Monilinia* spp. after the harvest

Beside fruits affected with brown rot, symptomless fruits were also collected at the moment of harvest in order to monitor brown rot development during storage. Totally 284 fruits from location A and 133 fruits from location B were collected in 2014. In 2015, 100 fruits from location A and 100 fruits from location B were sampled. Dynamics of brown rot development was monitored according to EPPO (2002). Fruits were kept in cold storage for five days after the harvest, and transferred to a chamber on a room temperature. Incidence of brown rot was assessed after eight days, 14 days and 21 days of storage.

Identification of *Monilinia* species

Population structure of *Monilinia* species on symptomatic fruits collected at the moment of harvest and on asymptomatic fruit collected and left in storage was determined in the Laboratory for mycology at the Institute for Plant Protection

Table 1. Brown rot (*Monilinia* spp.) incidence (%) on fruits at different assessment periods

Year	Trial location	Moment of harvest	After harvest (8 days)	After harvest (14 days)	After harvest (21 days)
2014	Location A	-	4.2	11.9	20.3
	Location B	2.9	70.6	85.3	92.6
2015	Location A	-	-	-	-
	Location B	-	-	-	8.0

– CCAFRA. Small pieces of *Monilinia* spp. mycelium developed on fruits were taken with a sterile needle and transferred to potato-dextrose agar (PDA). Three transfers were done from each fruit. PDA plates were incubated for 8-12 days at 22°C in 12 h light/12 h dark regime. *Monilinia* species were identified according to the morphology in culture, following descriptions of Lane (2002) and EPPO (2009), and population structure was determined.

Results

Monitoring of *Monilinia* spp. in the field

On the flowers, there were no visible symptoms of blossom blight at both locations and in both years. Symptoms of brown rot on fruits were neither recorded in assessment on fruits 13 days (2014) or 10 days (2015) before the harvest. In 2014, the first infection with *M. fructicola* on PinovaSoft® application was recorded on 25 April, and three more infections were recorded up until the first assessment on fruits two weeks before the harvest (1 May, 27 May and 24 June). In 2015, the first infection with *M. fructicola* on PinovaSoft® application was recorded on 14 April. Ten more infection moments were recorded up until 10 days before the harvest (26 May, 31 May, 16 June, 20 June, 28 June, 9 July, 12 July, 13 July, 15 July and 16 July).

The first and the only symptoms of brown rot on fruits were recorded on location B in 2014 (Table 1). No symptoms of brown rot in the field were recorded on location A in 2014, and no symptoms in the field were recorded at all in 2015. During 2014, totally seven infection events with *M. fructicola* were recorded during the vegetation period (those previously mentioned and on 4 July, 9 July and 15 July 2014). During 2015, even 15 infection events with *M. fructicola* were recorded (those previously mentioned and on 17 July, 19 July, 24 July and 25 July).

Monitoring of *Monilinia* spp. after the harvest

On the fruits collected in 2014, brown rot developed on fruits from both locations (Table 1). However, development of the disease was different on fruits from different experimental locations. On fruits from location B, almost 71% of fruits were found to be affected with brown rot after only 8 days of storage. On fruits from location A, only 4% of fruits with brown rot were recorded at the same assessment date. After three weeks of storage, brown rot developed on almost 93% of stored fruits from location B. At the same time, the incidence of brown rot on fruits from location A was approximately 20%.

In 2015, the development of brown rot during the storage was very weak. On fruits from location A, brown rot was not recorded at all up until the last assessment 21 days after harvest.

Population structure of *Monilinia* spp. on peach fruits

All three *Monilinia* species were determined in all assessments when fruits with brown rot symptoms were present. In 2015, *M. fructicola* was found on all 12 fruits on which brown rot developed, from location B and in assessment 21 days after the harvest. *M. laxa* and *M. fructigena* were found only on one fruit each, and these were mixed infections with *M. fructicola*. In 2014, population structure of *Monilinia* spp. on fruits was more diverse (Table 2). All three *Monilinia* species, *M. fructicola*, *M. laxa* and *M. fructigena* were found in all assessments when brown rot was recorded. In the field, in 2014 on location B, *M. fructigena* was found in relatively highest incidence (17 of 40 fruits), while *M. laxa* and *M. fructicola* were present in relatively similar proportions. Similar shares of all three *Monilinia* species were found 21 days after the harvest on fruits from location B. On fruits from location A, *M. laxa* was detected on 14 out of 30 fruits with brown rot and was relatively the most abundant, but neither species can be considered prevalent.

Table 2. *Monilinia* spp. population structure (% of isolates) in 2014 at different assessment periods on fruits affected with brown rot

Trial location	*Monilinia* species	Moment of harvest	After harvest (21 days)
Location A	*M. fructicola*	-	29
	M. laxa	-	47
	M. fructigena	-	24
Location B	*M. fructicola*	27	28
	M. laxa	30	34
	M. fructigena	43	38

Discussion

Blossom blight of peach can be caused by *M. fructicola* and *M. laxa* (Byrde and Willetts, 1977). Since the forecast model for *M. fructicola* in 2014 and in 2015 did not record any infections during the flowering period, which was confirmed by visual inspections, it is obvious that conditions for infections with *M. laxa* were not favourable. In fact, *M. fructicola* and *M. laxa* have similar requirements for conidial germination in relation to temperature and water activity (Casals et al., 2010). The results show that combining the forecast model for *M. fructicola* and *M. laxa* could lead to more efficient and rational fungicide treatments in peach orchards during the flowering period.

Brown rot symptoms were not visible on fruits two weeks before the harvest in both years and on both locations, even though the forecast model showed that several *M. fructicola* infections potentially occurred till the moments of assessment. It is evident that the number of recorded infections did not show any differences in the field, despite the fact that two times more infections with *M. fructicola* were recorded in 2015 than in 2014, 15 and 7, respectively. Development of brown rot on fruits in later stages, especially after harvest, showed that the infections happened, but were latent. Infections of *Monilinia* spp. on fruits are often latent at the moment of harvest (Thomidis, 2017; Byrde and Willetts, 1977). Brown rot can develop on fruits after the harvest, which was confirmed especially in 2014. From only about 3% of fruits affected at the moment of harvest on location B, brown rot incidence increased to 70% after only eight days of storage. After 21 days, brown rot developed on 93% of stored fruits. Such increase shows that fungicide treatments during the ripening of fruits, three to one week before the harvest, can be considered justified and necessary. However, this also shows that the forecast model might have a limited significance as a support tool for decision whether to apply fungicide close to the harvest. The number of infection events during the vegetation for *Monilinia* spp. may be less relevant in assessing the risk of brown rot. Despite higher number of recorded infections in 2015, brown rot incidence after the harvest was much higher in 2014 than in 2015. Beside differences between two years, differences in post-harvest brown rot on fruits from different locations was also obvious. Experimental locations A and B were relatively nearby (about 200 m air distance), and were within the same orchard, and this is showing the importance of micro-locations on brown rot development. The occurrence of brown rot may drastically increase or decrease depending on the inoculum (Holtz et al., 1998; Hong et al., 1997). Considering differences in brown rot incidence and development on fruits between two experimental locations, it may be presumed that *Monilinia* spp. inoculum is higher on location B, and it might favour stronger disease pressure. Also, it is possible that the inoculum of *Monilinia* spp. on location A appeared later during the vegetation period, or it was more distant. One peach fruit infected with *M. fructicola* can produce 3.5 to 11.7 million conidia during the vegetation (Hong et al., 1997). If conditions for *Monilinia* spp. infection are favourable and conductive for the development of brown rot, even a smaller number of infected fruit on a tree or on the ground may lead to the difference in post-harvest brown rot incidence. On location B, about 3% of fruits with brown rot were found at the moment of harvest. These fruits might have served as a source of inoculum, which was not present in such form on location A. Distinctive differences in brown rot development on stored fruits from the two experimental locations (20.3 and 92.6% after 21 days) may be in relation to the presence of fruit brown rot presence during the vegetation on location B, but not on location A.

Low incidence of *Monilinia* spp. on stored fruits in 2015 indicates that the disease pressure in the field was much lower comparing it to 2014 season. Different weather conditions in 2014 and 2015 could explain the difference in disease incidence. According to the meteorological data, 2014 was extremely rainy year during the vegetation period. On the other hand, spring and summer of 2015 were generally hot and arid in the continental part of Croatia. Dependence of brown rot incidence on climatic conditions during the season is known and well documented (Cvjetković, 2010; Holtz et al., 1998; Ogawa et al., 1995).

In 2014, the population structure of *Monilinia* spp. found on fruits showed relatively similar appearance of all three *Monilinia* species. *M. fructicola* comprised approximately one-third of the isolates, on fruits from both locations. The results show that this invasive alien species has adapted to agro-climatic conditions in Croatia, and has become a common part of *Monilinia* spp. population in the orchard. Comparing it to some other areas of the world, the prevalence of *M. fructicola* found in the present study is not high. In China in a natural range of *M. fructicola* occurrence, this species comprised even 93% of *Monilinia* spp. isolates on stone fruits, with only 4.8% of *M. fructigena* and 2% of *M. laxa* (Zhu et al., 2011). In Europe, the share of *M. fructicola* on stone fruits is lower (Hrustić et al., 2015; Papavasileiou et al., 2015; Poniatowska et al., 2013).

Although brown rot incidence during 2015 was very low, it is interesting to note that *M. fructicola* was found on all 8% of fruit on which brown rot developed in that year. Low brown rot incidence and only two years of monitoring are not allowing conclusions on the eventual shift of *Monilinia* species within natural populations, but it may possibly happen in a long-term period. Hrustić et at. (2015) recorded an increase in *M. fructicola* incidence among *Monilinia* species on stone fruit in the neighbouring Serbia, and are stating that this may indicate a change in population structure of brown rot pathogens.

In the present study, *M. fructigena* was found in relatively high incidence on fruits. Such results are contrary to Cvjetković (2010), who stated that this species is rare on peach fruits. *M. fructigena* incidence also differs from similar results in other European countries. This species comprised only 3% of the isolates from stone fruits in Serbia (Hrustić et al., 2015), and it was not found at all in Greece (Papavasileiou et al., 2015). *M. fructicola* was confirmed to be more aggressive species on peach than *M. laxa* and *M. fructigena* (Villarino et al., 2016), but the possible shift in *Monilinia* spp. populations on stone fruits in Croatia will depend on many factors.

Conclusions

Brown rot incidence on peach may extremely vary between the seasons. Utility of forecast model as a tool for decision support on whether to apply fungicides or not is limited, as *Monilinia* infections are often latent. Despite the low disease incidence in the field, brown rot may develop intensively on fruits during the storage. A history of brown rot pressure within a certain location should remain the base for decision on control strategies, and the use of a model forecasting infection events can be only an additional support. *M. laxa*, *M. fructigena* and *M. fructicola* can appear on peach fruit with similar frequencies. Results are indicating that common measures against indigenous *M. laxa* and *M. fructigena* within integrated pest management on peach may be suitable for the control of invasive *M. fructicola*. Considering the established control measures against *M. laxa* and *M. fructigena* in Croatia, it could be assumed that the overall impact of *M. fructicola* on Croatian stone fruit production could be relatively low. This is in line with the conclusion of EFSA (2011), stating that no increased fungicide treatments would be needed to control brown rot in stone fruits after the introduction of *M. fructicola* in Europe.

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