CZECH MYCOL. 63(1): 77–82, 2011

Endophytic fungi in branches of sour cherry trees: a preliminary study

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Hortová B., Novotný D. (2011): Endophytic fungi in branches of sour cherry trees: a preliminary study. – Czech Mycol. 63(1): 77–82.

Sour cherry trees (*Prunus cerasus* L.) harbour various fungal groups, including endophytes. Branches of two different cultivars of sour cherry trees (Újfehértoi Fürtös, Érdi Bötermö) were sampled in May and July 2010 at two study sites in the Czech Republic. *Alternaria alternata* and coelomycete sp. 1 were the dominant species in branches collected at one site. *Aureobasidium pullulans* was the most frequent fungal species in branches from the other site. Both species were dominant in May and July.

Key words: *Prunus cerasus*, Czech Republic, mycobiota, endophytic fungi.

Hortová B., Novotný D. (2011): Endofytické houby větví višní: předběžné výsledky. – Czech Mycol. 63(1): 77–82.

Višeň obecná (*Prunus cerasus* L.) hostí široké spektrum asymptomaticky žijících mikroskopických hub, tzv. endofytů. Tyto houby nebyly u višní na území ČR dosud zkoumány. V roce 2010 byly odebírány vzorky větví ze dvou různých odrůd višní (Újfehértoi Fürtös, Érdi Bötermö) ze dvou různých lokalit (Těšetice – jižní Morava, Lestkov – východní Čechy). Odběry probíhaly v květnu a červenci. U vzorků větví odebraných v Těšeticích byly dominantní druhy *Alternaria alternata* a coelomycet sp. 1. U vzorků větví pocházejících z Lestkova se nejčastěji vyskytoval druh *Aureobasidium pullulans*. Uvedené druhy byly dominantní v obou odběrech na dané lokalitě.

INTRODUCTION

Endophytes are common in different plants and reside inside healthy plant tissues without producing any disease symptoms (Larrán & Mónaco 2010). Fungal endophytes have been reported from agricultural commodities such as wheat (Larran et al. 2002a), soybeans (Larran et al. 2002b), tomatoes (Larran et al. 2001), and bananas (Photita et al. 2004). Some are very specific and colonise almost exclusively one host, while others are present in many hosts at varying degrees of frequency (Kowalski & Kehr 1996). The composition of endophytic mycobiota of fruit trees can be diverse depending on the tree species studied, but the degree of diversity of endophytic genera and species in fruit trees is similar (Dugan & Roberts 1994).

Fungal endophytes of fruit trees have been investigated less frequently than those of forest trees. So far research on fungal endophytes of fruit trees has focused mainly on aerial parts especially leaves, branches and fruits (Novotný 2007). Dugan & Roberts (1994) studied the colonisation of cherry fruits by endophytes, while Camatti-Sartori et al. (2005) investigated endophytic fungi of apple and Johnston (1994) those of apple and kiwifruit. In the Czech Republic only endophytes of apple trees (Novotný 2007), oaks (Novotný 2003), vine (Šilhánová 2006) and elms (Dvořák et al. 2006) have been studied to date. The sour cherry is one of the most popular temperate fruit crops. It is mainly used for processed products such as pie filling, jam and cherry brandy (Dirlewanger et al. 2009). No information concerning endophytic mycobiota of sour cherry trees is available.

The investigation of endophytic fungi has several practical aspects. Endophytes may adapt to their hosts and be antagonists for their pathogens and, accordingly, they could reduce, suppress or induce resistance against them (Larrán & Monaco 2010). Many endophytic microorganisms are able to produce compounds of biotechnological value as antibiotics and antitumoral drugs (Museti et al. 2007). Liquid extracts from endophyte cultures have been found to inhibit the growth of several species of plant pathogenic fungi (Liu et al. 2001, Kim et al. 2007).

The aim of the present, preliminary study is to contribute to the knowledge of endophytic mycobiota of sour cherry trees branches in the Czech Republic.

MATERIALS AND METHODS

Eighty asymptomatic approximately four-years olds branches of sour cherry trees were collected from two locations (conventional orchards in the villages of Těšetice – 48°53'19.842" N, 16°9'29.527" E, South Moravia and in Lestkov – 50°34'50.569" N, 15°15'1.99" E, East Bohemia) in the Czech Republic. Samples were collected from two cultivars (Érdi Bötermö, Újfehértoi Fürtös). Ten healthy randomly selected trees of each cultivar were taken. Branches were sampled in May (Těšetice: 17 May 2010, Lestkov: 31 May 2010) and in July (Těšetice: 12 July 2010, Lestkov: 15 July 2010). Two branches of each selected tree at each sampling date were taken.

Samples of the branches were brushed under running water and their surface sterilised [96% ethanol 1 min., sodium hypochlorite (NaClO) 2 min., 96 % ethanol 30 s, sterile water 15 s], cut into pieces of approx. 3–5 × 3–5 × 1–2 mm. Five pieces per branch were placed in one Petri dish each, containing 2% malt extract agar. Dishes were incubated at 22 °C during four weeks. Identifications were made in the original dishes or when this was not possible, fungi were incubated for further study on 2% malt extract agar. The colonisation frequency (CF) of an endophyte species was calculated as the number of segments colonised by fungi divided by the total number of segments incubated.

RESULTS AND DISCUSSION

Fifteen species of fungi (including sterile mycelia) were recorded in branches of sour cherry trees (Tab. 1).

In Těšetice we recorded thirteen endophytic species. *Alternaria alternata* and coelomycete sp. 1 were the dominant species in May. The incidence of these was almost similar in both cultivars (*Alternaria alternata* – 40 %, coelomycete sp. 1–27 %). In July the incidence was lower. Dominant species were again *Alternaria alternata* (in cultivar Újfehértoi Fürtös it was 13.5 %, in cultivar Érdi Bötermö 6 %) and coelomycete sp. 1 (Újfehértoi Fürtös 15.5 %, Érdi Bötermö 3.5 %).

In Lestkov we also recorded thirteen species of fungi. *Aureobasidium pullulans* was the most frequent. For both cultivars in May the incidence was similar (*A. pullulans* colonising 20 % of the segments). In July the incidence was lower, with the dominant fungal species in both cultivars being *Cladosporium herbarum* (9 %).

Endophytic mycobiota of aerial organs of sour cherry trees have not yet been investigated, but we can compare our results with at least those reported for other fruit tree species. Dugan & Roberts (1994) investigated the mycobiota of cherry fruits and isolated most frequently *Alternaria*, *Cladosporium* and *Aureobasidium*. Novotný (2007) studied the endophytic fungi of apple trees and recorded most frequently *Pleurophoma cava*, *Alternaria alternata*, *Aureobasidium pullulans*, *Seimatosporium* cf. *lichenicola*, *Phomopsis* cf. *mali* and *Microsphaeropsis* sp. In the present study *Alternaria alternata*, *Aureobasidium pullulans*, *Cladosporium herbarum* and coelomycete sp. 1 were the most frequent fungal species. Thus the endophytic mycobiota of apple, cherry and sour cherry are similar. *Alternaria*, *Cladosporium* and *Aureobasidium* have been isolated worldwide. These species are known as opportunistic pathogens affecting many cultivated plants in the field and during postharvest storage of fruit and vegetables (Guo et al. 2004, Jones & Aldwinckle 1990) or as epiphytes on the surface of leaves or as soil saprotrophs also (Domsch et al. 2007, Schubert et al. 2007).

Tab. 1 Endophytic fungi recorded in sour cherry branches from south Moravia and east Bohemia (cultivars of sour cherry trees UF – Újfehértoi Fürtös, EB – Érdi Bötermö)

Fungal species	**Těšetice (south Moravia)**				**Lestkov (east Bohemia)**			
	May		July		May		July	
	UF (%)	EB (%)	UF (%)	EB (%)	UF (%)	EB (%)	UF (%)	EB (%)
Alternaria alternata (Fr.) Keissler	40	38	13.50	6	11	10	1	5
Aureobasidium pullulans (de Bary) G. Arnaud		1.5	1	4	19	21.5	3	4
Botrytis cinerea Pers	1	0.5		11		3		
Cladosporium cladosporioides (Fresen.) G. A. de Vries			3	5			2	7
Cladosporium herbarum (Pers.: Fr.) Link		1	1		4.5	1	8	9
coelomycete sp. 1	27	26	15.5	3.5				1
coelomycete sp. 2	8	8		1	3	1		
coelomycete sp. 3	3.5							
Epicocum nigrum Link	2	1	0.5		10	3	2	2
Pezicula sp.					1			
Sarcinomyces sp.	2	4			5			
sterile grey mycelium	4	3	6	0.5	5.5	2	4	4
sterile beige mycelium	5	1	1	3.5	3	8	8	0.5
sterile dark mycelium			1					
sterile green mycelium			5			2	3	

Alternaria alternata and coelomycete sp. 1 were the dominant species in Těšetice, whereas *Aureobasidium pullulans* and *Cladosporium herbarum* dominated in Lestkov. Less frequently isolated species encountered at both localities were *Botrytis cinerea*, *Cladosporium cladosporioides*, coelomycete sp. 1, coelomycete sp. 2, *Epicocum nigrum*, *Pezicula* sp., *Sarcinomyces* sp. and a sterile mycelium. The endophytic mycobiota composition was very similar at both localities, but considerable differences were seen in their frequency.

Alternaria alternata was most frequently found in this study. This species has been commonly isolated as an endophyte from a wide range of plants. It has been recorded from several crops including apple (Johnson at al. 1994, Serdani et al. 1998) and sweet cherry (De Vries–Paterson et al. 1991, Serdani et al. 1998). *Alternaria* spp. are known to cause core rot of fruit trees and postharvest losses in fruit (Dugan & Roberts 1994).

Aureobasidium pullulans, frequently recorded in the present study, may cause apple russet, which can deteriorate the visual appearance and marketability of fruits. However, this species is known as an endophyte of fruit trees and is used as a potential biocontrol agent against post-harvest pathogens (*Botrytis cinerea*, *Penicillium expansum*, *Pezicula malicorticis*) (Leibinger et al. 1997, Granado et al. 2008).

ACKNOWLEDGEMENTS

The work was supported by the Grant Agency, Faculty of Agrobiology, Food and Natural Resources, Czech University of Agriculture (Project No. 3119/21180/1312) and MZE0002700604 from the Ministry of Agriculture of the Czech Republic.

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