First report on *Monilinia fructicola* in the Slovak Republic

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Abstract

Ondejková N., Hudecová M., Bacigálová K. (2010): **First report on *Monilinia fructicola* in the Slovak Republic**. Plant Protect Sci., **46**: 181–184.

The occurrence of *Monilinia* on stone and pome fruit trees in Slovakia was studied. Commonly distributed species *Monilinia laxa*, *M. fructigena*, and American species *M. fructicola* were determined by the methods used. *M. fructicola* was identified on the fruits of peach and nectarine imported to our country and on domestic plum fruits as well. To our knowledge, this is the first report on *M. fructicola* in Slovakia and a new member for Slovak mycobiota.

Keywords: *Monilinia laxa*; *Monilinia fructigena*; *Monilinia fructicola*; new record

Phytopathogenic micromycetes of the genus *Monilinia* (*Monilia* anamorph stage) are responsible for blossom and twig blight, formation of cancers and predominantly brown rot of stone and pome fruit crops. *M. fructigena* Honey and *M. laxa* (Aderh. et Ruhland) Honey occurs in Europe very frequently. *M. fructicola* (G. Winter) Honey has been restricted to Australia, South Africa, North and South America until now and is the European quarantine listed organism (Anonymous 1992). However, it has recently been introduced into Europe, first identified in France (Anonymous 2002a), consequently in Austria (Anonymous 2002b) and is further spreading into other countries where it was identified on both imported and domestic fruits (Bosshard *et al*. 2006; Petroczy & Palkovics 2006; Duchoslavová *et al*. 2007). Due to the economic impact of all species and spreading of the non-European one, the national survey of *M. fructicola* is conducted on the basis of a European directive. Besides phytosanitary measures taken in the case of outbreak, the results of this survey contribute to the knowledge of Slovak mycobiota.

MATERIAL AND METHODS

The *Monilinia* species were searched in naturally infected tissues of fruits, blossoms and twigs. Tested samples came from domestic orchards, private gardens, fruit nurseries, natural sites of Slovakia, and a few samples came from markets and were predominantly imported to the country. Most of the samples were collected in cooperation with phytosanitary inspectors. During the survey in 2005–2008, a total number of 221 samples coming from 89 localities were analysed. Laboratory-based techniques combining cultural characteristics, morphological data and molecular-genetic diagnostic methods were used for the accurate identification. The growth characteristics were studied on 2% PDA (Biomark Laboratories, Pune, India) (cultivation for 4 days at 22°C in darkness, subsequently subcultivation in 12 h light:12 h dark regime). Colour and colony margins, formation of rosettes and their lobes, sporulation, size, colour and shape of conidia and germination and branching of germ tubes were studied. Van Leeuwen & van Kesteren (1998),

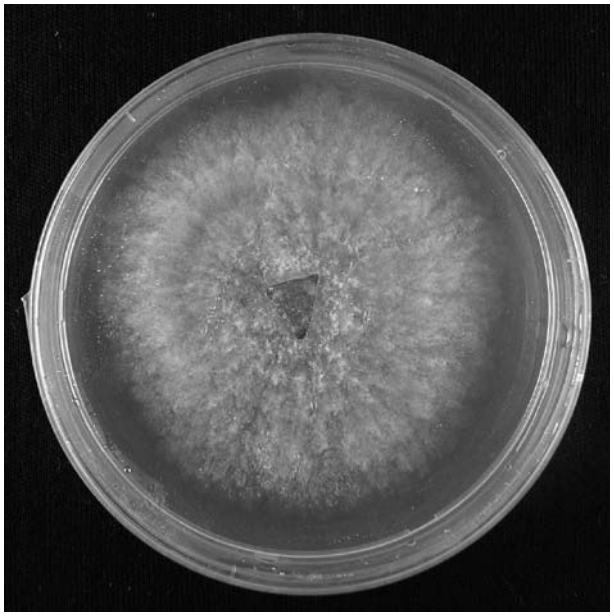

Figure 1. Colony of *M. fructicola* isolate grown on PDA – 7 days old colony

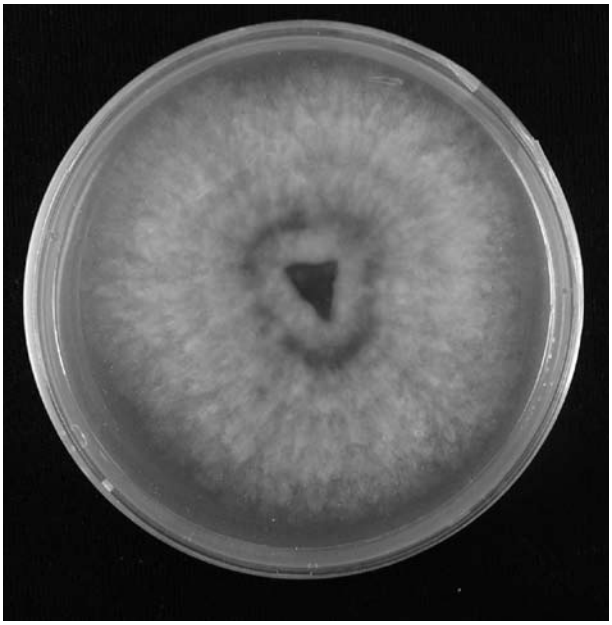

Figure 2. Colony of *M. fructicola* isolate grown on PDA – 7 days old colony, reverse

De Cal & Melgarejo (1999), Lane (2002) and Baayen *et al*. (2003) were used for determination. Molecular-genetic identification was carried out by polymerase chain reaction (PCR). DNA was isolated by standard isolation kits and specific sets of PCR primers according to Ioos and Frey (2000) for *M. fructicola* and Hughes *et al*. (2000) for *M. laxa* and *M. fructigena* were used.

RESULTS

In the period of 2005–2008, the presence of *Monilinia* species was recorded on 162 samples coming from 67 localities. Infection by single species *M. laxa* was identified on 65% of samples and *M. fructigena* on 27% of samples. Mixed infection of *M. laxa* and *M. fructigena* on one fruit was determined as well (6% of samples). Four samples of stone fruit (2%) were infected by *M. fructicola,* thereof one sample was of domestic origin and three samples were imported.

Monilinia fructicola was identified in the anamorph stage as *Monilia fructicola* and caused the rot of fruits with profuse sporulation forming grey pustules usually in concentric or random distribution. Two isolates from imported peaches came from Greece and Spain, one isolate from nectarine came from Greece and the domestic isolate from plum came from Spišský Štiavnik. All identified isolates are kept in the diagnostic laboratory of Central Control and Testing Institute of Agriculture in Bratislava.

On PDA the fast-growing grey mycelium with entire colony margin was formed, showing no growth rhythm (Figures 1 and 2). A growth rate of the fungus on PDA was 8.7–9.2 cm per ten days. Sporulation of this species on PDA was very

Figure 3. Colony of *M. fructicola* isolate grown on PDA – profuse sporulation

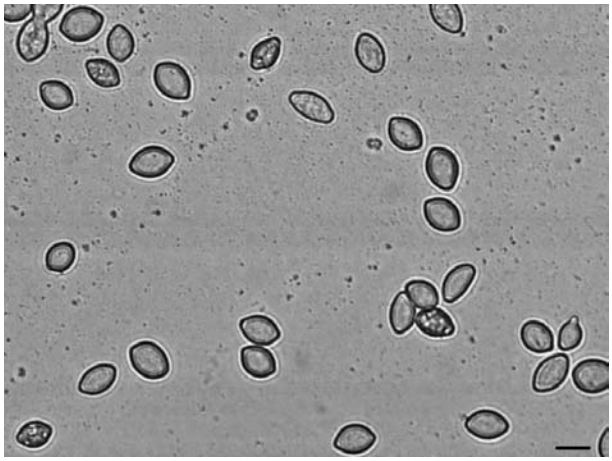

Figure 4. Conidia (bar = 10 µm)

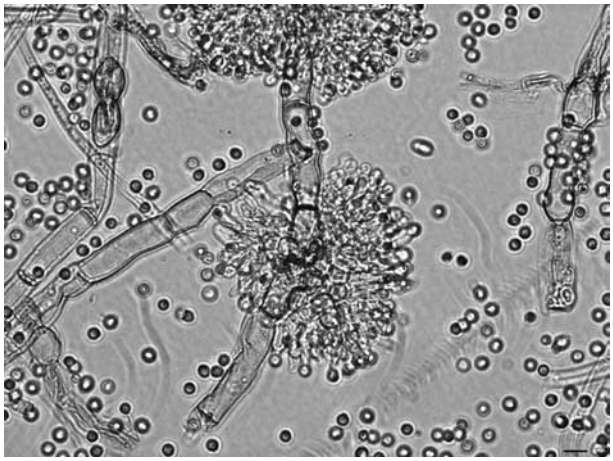

Figure 5. Formation of microconidia on 30 days old colony (bar = 10 µm)

profuse, usually in concentric rings or over the entire surface of the colony (Figure 3).

Conidia on PDA were 11.09–19.12 × 7.10–12.62 µm. Conidia formed in chains were ellipsoid, ovoid and limniform, some with truncate ends (Figure 4). In old colonies, discoid sclerotia were formed as well as globose hyaline microconidia (3 µm in diameter) produced by bottle-shaped, often asymmetric phialides (Figure 5). The formation of microconidia was noticed in whole colonies. The specific primer set for *M. fructicola* yielded a diagnostic amplicon of 350 bp (Figure 6).

The results obtained in our study are mainly fundamental, however, the identification of *M. fructicola* on domestic fruits in Slovakia and on imported fruits is an important contribution to the knowledge of Slovak mycobiota, confirming the spread of this fungus to the European continent.

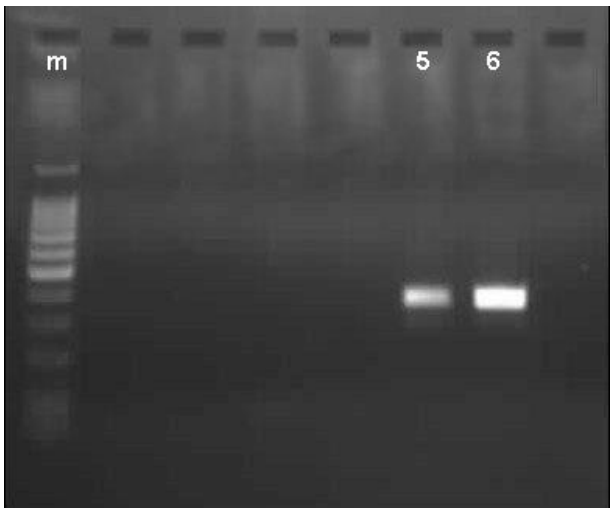

Figure 6. Amplification products generated by specific pairs of primers for *M. fructicola,* m – 100 bp ladder, lane 5 – fragment obtained from *M. fructicola*, lane 6 – positive control

Acknowledgements. We would like to express our thanks to Jana Duchoslavová from the Division of Diagnostics, State Phytosanitary Administration, Olomouc, Czech Republic, for help with molecular diagnostics and to all technical staff for their excellent assistance.

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Received for publication March 16, 2009
Accepted after corrections August 15, 2010

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