

First report on *Monilinia fructicola* in the Slovak Republic

NADEŽDA ONDEJKOVÁ¹, MICHAELA HUDECOVÁ¹ and KAMILA BACIGÁLOVÁ²

¹Section of Diagnostics, Central Control and Testing Institute of Agriculture in Bratislava, Bratislava, Slovak Republic; ²Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovak Republic

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),

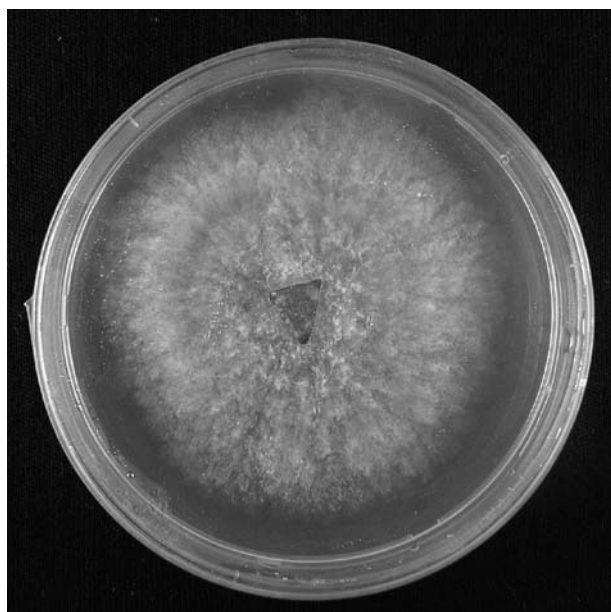


Figure 1. Colony of *M. fructicola* isolate grown on PDA – 7 days old colony

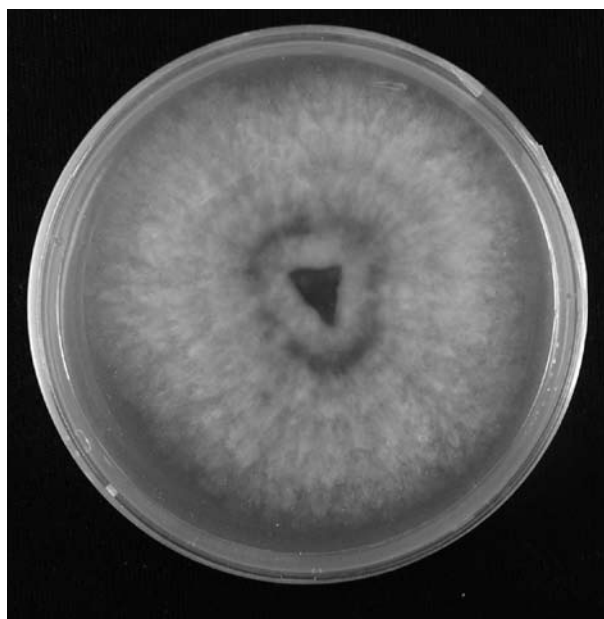


Figure 2. Colony of *M. fructicola* isolate grown on PDA – 7 days old colony, reverse

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.

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

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.



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

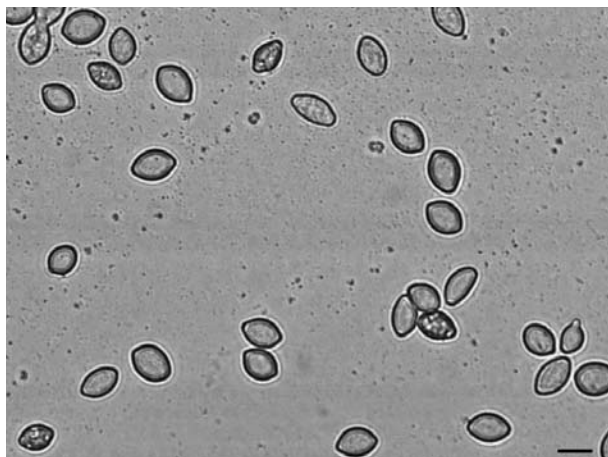


Figure 4. Conidia (bar = 10 µm)

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

Conidia on PDA were $11.09\text{--}19.12 \times 7.10\text{--}12.62$ 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



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

is an important contribution to the knowledge of Slovak mycobiota, confirming the spread of this fungus to the European continent.

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.

References

- ANONYMOUS (1992): *Monilinia fructicola* – Data sheet on quarantine pest. In: Quarantine Pests for Europe. CAB International, Wallingford: 530–535.
- ANONYMOUS (2002a): First report of *Monilinia fructicola* in France. EPPO Reporting Service 2002/003.
- ANONYMOUS (2002b): First report of *Monilinia fructicola* in Austria. EPPO Reporting Service 2002/170.
- BAAYEN R.P., PIETERS R.M., VAN LEEUWEN G.C.M., HUGHES K.J.D., LANE C. R. (2003): Diagnostic protocol for regulated pest – *Monilinia fructicola*. EPPO Bulletin, **33**: 281–288.
- BOSSHARD E., HILBER-BODMER M., SCHÄRER H.-J., BÜNTER M., DUFFY B. (2006): First report of the quarantine brown rot pathogen *Monilinia fructicola* on imported stone fruits in Switzerland. Plant Disease, **90**: 1554.
- DE CAL A., MELGAREJO P. (1999): Effects of long-wave UV light on *Monilinia* growth and identification of species. Plant Disease, **83**: 62–65.
- DUCHOSLAVOVÁ J., ŠIRŮČKOVÁ I., ZAPLETALOVÁ E., NAVRÁTIL M., ŠAFÁŘOVÁ D. (2007): First report of

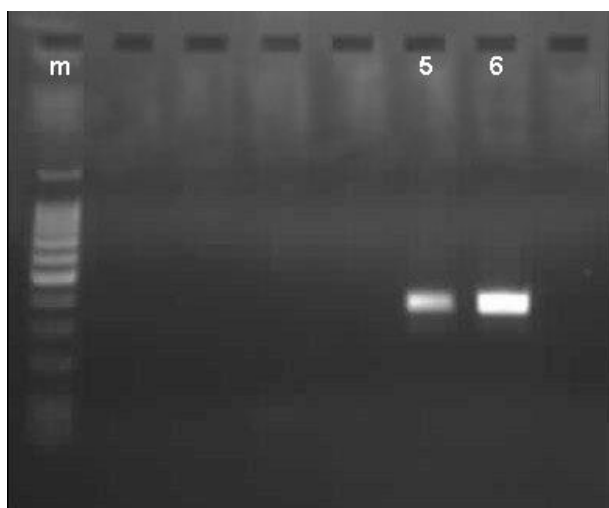


Figure 6. Amplification products generated by specific pairs of primers for *M. fructicola*, m – 100 bp ladder, lane 5 – fragment obtained from *M. fructicola*, lane 6 – positive control

- brown rot caused by *Monilinia fructicola* on various stone and pome fruits in the Czech Republic. Plant Disease, **91**: 907.
- HUGHES K.J.D., FULTON C.E., McREYNOLDS D., LANE C.R. (2000): Development of new PCR primers for identification of *Monilinia* species. EPPO Bulletin, **30**: 507–511.
- IOOS R., FREY P. (2000): Genomic variation within *Monilinia laxa*, *M. fructigena* and *M. fructicola*, and application to species identification by PCR. European Journal of Plant Pathology, **106**: 373–378.
- LANE C.R. (2002): A synoptic key for differentiation of *Monilinia fructicola*, *M. fructigena* and *M. laxa*, based on examination of cultural characters. EPPO Bulletin, **32**: 489–493.
- LEEUWEN VAN G.C.M., KESTEREN VAN H.A. (1998): Delineation of the three brown rot fungi of fruit crops (*Monilinia* spp.) on the basis of quantitative characteristics. Canadian Journal of Botany, **76**: 2042–2050.
- PETROCZY M., PALKOVICS L. (2006): First report of brown rot caused by *Monilinia fructicola* on imported peach in Hungary. Plant Disease, **90**: 375.

Received for publication March 16, 2009

Accepted after corrections August 15, 2010

Corresponding author:

RNDr. NADEŽDA ONDEJKOVÁ, Ústredný kontrolný a skúšobný ústav poľnohospodársky, Odbor diagnostiky, Hanulova 9/A, 844 29 Bratislava, Slovenská republika
tel.: + 421 269 204 436, e-mail: nadezda.ondejkova@uksup.sk
