INTRASPECIFIC VARIABILITY IN THE PHYTOPATHOGENIC FUNGUS *Monilinia laxa* (Aderh. & Ruhland) Honey

Tjaša GRIL[1], Branka JAVORNIK[2], Alenka MUNDA[3], Franci CELAR[4], Jernej JAKŠE[5]

[1,2,5]Biotechnical Faculty, Department of Agronomy, Centre for Plant Biotechnology and Breeding, Ljubljana
[3]Agricultural Institute of Slovenia, Plant Protection Department, Ljubljana
[4]Biotechnical Faculty, Department of Agronomy, Chair of Entomology and Phytopathology, Ljubljana

ABSTRACT

Brown rot fungi are found in most temperate regions in which apples, pears and stone fruits are grown. They have often caused considerable losses and damage to fruit crops and to the trees themselves. The group includes three species: *Monilinia laxa* (Aderh. & Ruhl.) Honey, *Monilinia fructigena* (Aderh. & Ruhl.) Honey and *Monilinia fructicola* (Wint.) Honey. They cause brown rot of stone and pome fruits, which result in considerable economic losses. A special form of the fungus, *M. laxa* f. sp. *mali,* is found only in apple, where it causes blossom wilt, spur-kill and canker. It has not yet been clearly confirmed whether this is a special form of the fungus or merely a race. There are some hints that it could even be a new species, *Monilinia mali*. *M. fructicola* is a quarantine pathogen in the EU (Directive du conseil 77/93/CEE, 1976; OEPP, 1996) while *M. fructigena* has the same status in the USA (Code of Federal Regulation, 1996) and Australia (Commonwealth Department of Health, 1984). *Monilinia laxa* f. sp. *mali* has a similar status in Australia. It is thus important to have reliable methods for identifying the pathogen. Distinguishing these fungus species from the genera *Monilinia* has been done for many years on the basis of morphological and cultural differences. Some of the morphological aspects are identical, so these methods are not reliable for routine work. PCR diagnostic methods have been mainly used for distinguishing between *M. fructicola* and the two other species and between *M. laxa* and *M. fructicola*, respectively. In some cases these methods are not reliable because of intraspecific variability. An alternative possibility for detecting intraspecific variability is AFLP (Amplified Restriction Length Polymorphism). AFLP method optimization for fungus *M. laxa* will be further presented.

Key words: AFLP (Amplified Restriction Length Polymorphism), forma specials intraspecific variability, *Monilinia laxa*, *Monilinia laxa* f. sp. *mali*

[1] univ. dipl. inž. agr., Jamnikarjeva 101, SI-1111 Ljubljana
[2] red. prof., dr., prav tam
[3] dr., Hacquetova 17, SI-1000 Ljubljana
[4] izr. prof., dr. agr. znan., Jamnikarjeva 101, SI-1111 Ljubljana
[5] doc., dr., prav tam

IZVLEČEK

ZNOTRAJVRSTNA VARIABILNOST FITOPATOGENE GLIVE *Monilinia laxa*

Med pomembnejše bolezni jabolk sodijo glive iz rodu *Monilinia*, ki jih označujemo s skupnim terminom »glive rjave gnilobe«. Znotraj rodu so znane tri vrste, ki povzročajo gnilobo plodov, sušenje cvetov in poganjkov ter rakavost vejic pri rodovih *Prunus* (koščičarji) in *Malus* ter *Pyrus* (pečkarji), kar se odraža v precejšnji gospodarski škodi. To so *Monilinia laxa* Aderhold & Ruhland, *Monilinia fructigena* Honey in *Monilinia fructicola* (Wint.) Honey. Specializirana forma glive, *M. laxa* f. sp. *mali*, najdena samo na jablani, povzroča sušenje cvetov, odmiranje poganjkov in raka. O tem ali gre zares za specializirano formo ali samo za raso ni v literaturi zanesljivih podatkov. Obstajajo celo namigi, da bi lahko šlo celo za novo vrsto, *Monilinia mali*. *M. fructicola* je v Evropski skupnosti uvrščena med karantenske patogene organizme (Directive du conseil 77/93/CEE, 1976; OEPP, 1996), medtem ko ima *M. fructigena* isti status v ZDA (Code of Federal Regulation, 1996) in Avstraliji (Commonwealth Department of Health, 1984). V Avstraliji ima podoben status tudi *Monilinia laxa* f. sp*. mali*. Za naštete države je zanesljiva identifikacija teh patogenov izredno pomembna, kakor tudi za države, ki izvažajo svoje pridelke v te države. Omenjene vrste gliv iz rodu *Monilinia* so vrsto let ločevali na podlagi morfoloških in rastnih razlik. Nekateri morfološki kriteriji se pri posameznih vrstah med seboj prekrivajo, zato klasične metode niso dovolj zanesljive za rutinsko uporabo. Na PCR temelječe diagnostične metode so bile uporabljene predvsem za ločevanje vrste *M. fructicola* od drugih dveh vrst oziroma za ločevanje vrste *M. laxa* od vrste *M. fructicola*. V nekaterih primerih tudi te identifikacijske metode niso povsem zanesljive, zaradi variabilnosti znotraj vrst. Ena izmed novejših možnosti za ugotavljanje polimorfizma znotraj vrste je molekulska metoda AFLP (dolžinski polimorfizem amplificiranih fragmentov), s katero bomo poskušali odkriti in potrditi razlike med izolati glive *M. laxa* iz koščičarjev in pečkarjev. V delu bo predstavljena optimizacija AFLP metode za omenjeno vrsto glive.

Ključne besede: AFLP (dolžinski polimorfizem amplificiranih fragmentov), forma specialis znotrajvrstna variabilnost, *Monilinia laxa*, *Monilinia laxa* f. sp. *mali*

1 INTRODUCTION

Three phytopathogenic fungi species within the *Monilinia* genus are known: *Monilinia fructigena* Honey, *Monilinia fructicola* (G. Winter.) Honey, and *Monilinia laxa* (Aderh. & Ruhl.) Honey. The latter has been known as a blossom and twig pathogen of stone fruits but has lately also been causing damage in apple tree orchards. A forma specialis of *Monilinia laxa* f. sp. *mali* Harrison{?} is also mentioned in the literature (1), which is sometimes recognized as the species *Monilinia mali* (2). *Monilinia laxa* f. sp. *mali* is thought to have developed from *Monilinia laxa* and infects apple trees.

Blossom blight during spring blossoming is the first sign of infection caused by *Monilinia laxa*. Blossom blight results in a reduction of the fruit set and also infection of the fruitlets. The infected tissue turns dark brown and (the) discoloration extends through all the flower parts, down the pedicel and into the young fruit. Infected leaves lose vigor, they dry up after a few days and they have a burned appearance. The fungus spreads from the floral parts through the peduncle into the twigs, in which infected tissue appears as brown, collapsed areas. Mycotoxins accelerate plant tissue decay. The shoot tips become hook-shaped and this symptom can easily be mistaken for other plant pathogens. Many orchards have been

destroyed due to incorrect identification of the pathogen. Under moist conditions, almost the entire surface of soft, ripe fruit is covered with conidial tufts or vegetative mycelium.
Monilinia laxa was found in Europe for the first time in 1933. There have recently been some reports of the fungus in India. Celar and Valič reported on the first finding of the pathogen in an apple orchard in Resje, Slovenia, in 1997 (3).
The special form of the fungus *M. laxa* f. sp. *mali* has not been examined in detail in any part of the world so far and intraspecific relationships have not yet been determined. It has not even been clearly confirmed whether it is a special form or a new species within the genus. In addition, the fungus causes symptoms very similar to those caused by the quarantine bacterium *Erwinia amylovora* (Burrill) Winslow *et al*., which is a significant problem in phytosanitary practice.
The purpose of the current study was to optimize a molecular method for analysing the phytopathogenic fungi *Monilinia laxa* and *Monilinia laxa* f. sp. *mali* from different hosts and to define their genetic diversity and relationship.

2 MATERIAL AND METHODS

- we collected 11 isolates of *Monilinia laxa* (table 1) from different host plants (stone and pome fruits) (Dr. Alenka Munda, KIS)

Table 1: Eleven isolates of *Monilinia laxa* used in this study

No.	Isolate	Host plant
1.	MLX 11 K	Apricot
2.	MLX 218 K	Apricot
3.	MLX 3 K	Apricot
4.	MLX 71 K	Plum
5.	MLX 21 K	Cherry
6.	MLX 53 P	Apple
7.	MLX 63 P	Apple
8.	MLX 64 P	Apple
9.	MLX 65 P	Apple
10.	MLX 215 P	Apple
11.	MLX 67 P	Apple

- we isolated genomic DNA from fungal mycelium from 3-4-day old liquid media with a standard CTAB buffer
- fluorescent AFLP (Amplified Fragment Length Polymorphism) analysis was carried out by using 9 different *Eco*RI/*Msp*I primers with two selective bases (detailed protocol is available from the author), electrophoregrams were visualized and analyzed by AlleleLocator 1.03,
- genetic similarities among individual isolates were calculated from an input binary matrix using Jaccard's coefficient of similarity. UPGMA was used to cluster them in different groups.

3 RESULTS AND DISCUSSION

All primer pair combinations selected for the analysis gave clear fragments (table 2). In the analysis of 11 *Monilinia laxa* isolates, the total number of amplified fragments was 968, of which 792 were polymorphic (81.82 %). The highest number of polymorphic fragments, 123, was amplified by primers E-GT + M-CG, the fewest, 51, by primers E-GT + M-GA. The

average percentage of polymorphism ranged from 62.20 % primer for the seventh primer combination to 99.15% for the ninth combination.

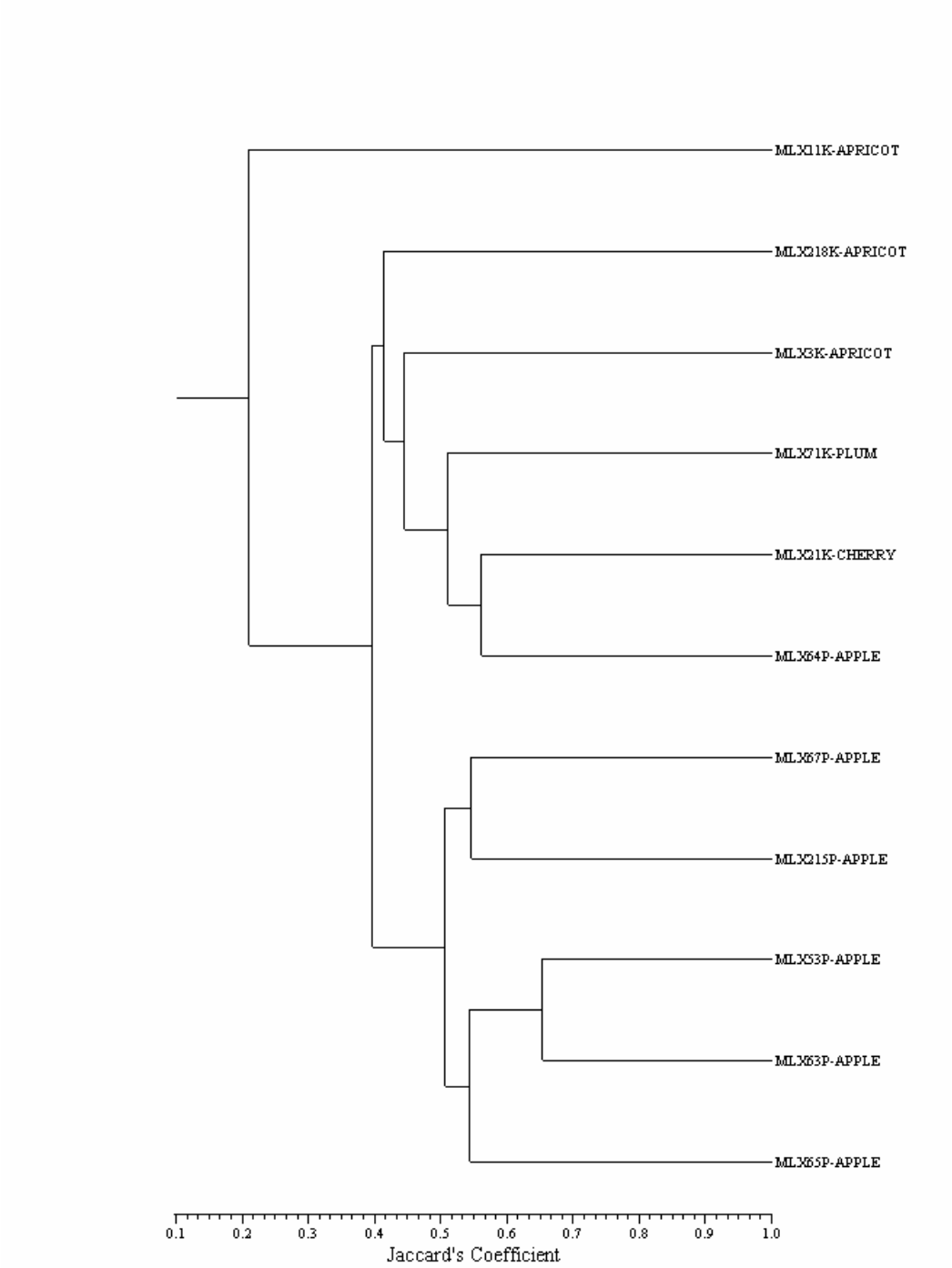

Figure 1: Dendrogram of 11 isolates of *Monilinia laxa* from different host plants based on AFLP analysis, using the Jaccard coefficient of similarity and UPGMA clustering

The lengths of amplified fragments longer than 50 bp, ranged from 488 bp (third combination) to 767 bp (first combination). Cluster analysis divided the *Monilinia laxa* isolates into two main groups. All isolates of the fungus from infected apple trees were genetically different from isolates of infected stone fruits, except MLX64P-JAB, an apple tree isolate which shows higher genetic similarities with the group of fungal isolates from stone fruit.
The first results of clustering the pathogen into groups (fig. 1) show a possible intraspecific variability of the fungus.

Table 2: Total number of AFLP fragments, number of polymorphic fragments, percentage of polymorphisms and length of amplified fragments, observed using 9 different primer pair combinations

No.	Primer pair combination	Scored number of amplified fragments	Number of polymorphic fragments	Polymorphisms (%)	Length of amplified fragments (>50bp)
1.	E-GT + M-CG	129	123	95,35	767
2.	E-GA + M-GT	92	67	72,83	693
3.	E-GA + M-CG	113	103	91,15	488
4.	E-GA + M-AT	101	72	71,29	747
5.	E-GA + M-TA	135	92	68,15	734
6.	E-GA + M-AG	118	100	84,75	660
7.	E-GT + M-GA	82	51	62,20	753
8.	E-AC + M-TA	118	117	99,15	714
9.	E-AC + M-GT	80	67	83,75	700

4 FURTHER WORK

In order to confirm and expand new findings on the examined pathogen we will:
- perform sampling from infected stone and pome fruit trees in intensive orchards in different regions in Slovenia
- collect and compare some isolates of the fungus from mycological collections in other parts of the world. Isolates from Japan and India are particularly interesting, where the presence of a special form of the fungus has been reported,
- compare the observed species *Monilinia laxa* with two other phytopathogenic species from the same genus (*Monilinia fructigena* and *Monilinia fructicola*)
- also attempt to elaborate a detailed fungal karyotype and measure the genome size of the pathogen.

5 REFERENCES

Byrde, R. J. W., Willetts, H. J. 1977. The brown rot fungi of fruit. Their biology and control. Oxford, UK: Pergamon, Press: 171 str.

Batra, L. R., Harada, Y. 1986. A field record of apothecia of *Monilinia fructigena* in Japan and its significance. Mycologia 78: 913-917.

Celar, F. Valič, N. 1999. Pojav glive *Monilinia laxa* f. sp. *mali* v Sloveniji. Zbornik predavanj in referatov 4. slovenskega posvetovanja o varstvu rastlin v Portorožu od 3. do 4. marca 1999: 485-488.