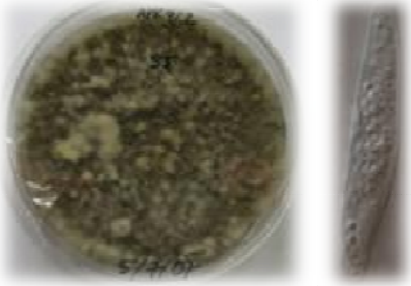
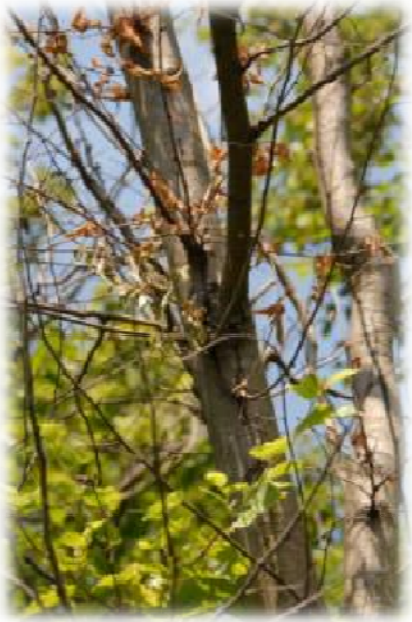
Vpliv neobičajnih vremenskih razmer na patogeno delovanje endofitnih gliv – primer črnega gabra na Krasu

dr. Barbara Piškur

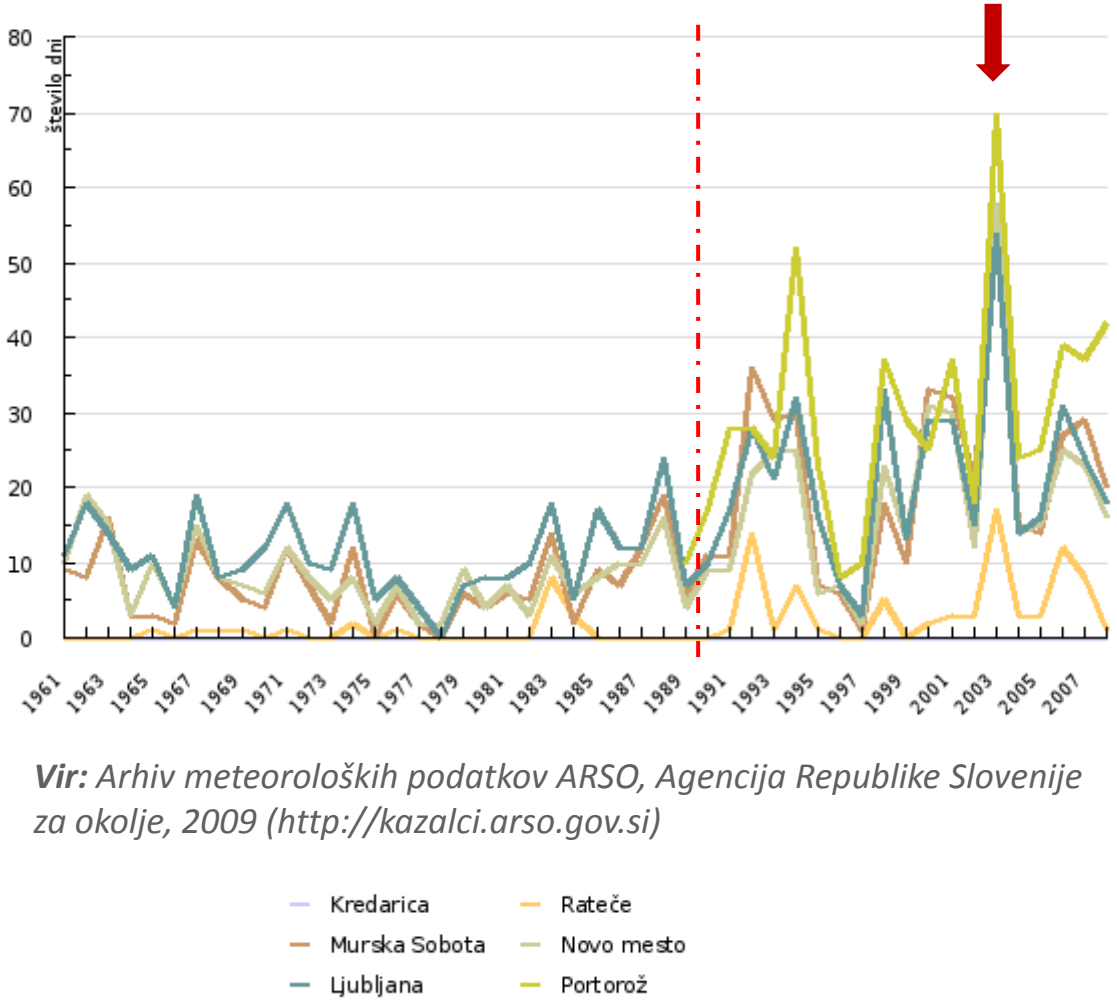
Oddelek za varstvo gozdov

Gozdarski inštitut Slovenije



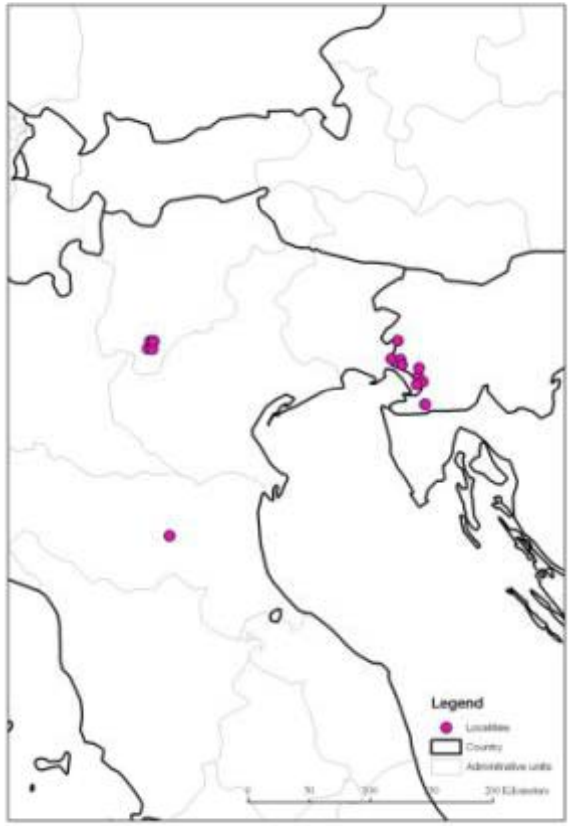


Število dni z najvišjo temperaturo vsaj 30°C



Vir: *Arhiv meteoroloških podatkov ARSO, Agencija Republike Slovenije za okolje, 2009 (http://kazalci.arso.gov.si)*

Sušenje črnega gabra



- neobičajno sušenje in odmiranje črnega gabra (*Ostrya carpinifolia*) na Krasu od l. 1997
- 2003 → ekstenzivna poškodovanost in mortaliteta črnih gabrov
- 2003 → neobičajne vremenske razmere:
  - visoke temperature
  - ekstremne suše
- 2003 → prizadetih 6.800 ha ; 50 % mortaliteta
- 2004 → 35 % mortaliteta, manjše območje

Simptomi



Okuženo drevo posledično odmre …

… ali pa nastane rak.

Nespolna trosišča gliv iz družine Botryosphaeriaceae na odmrli skorji.

V zimskem času – obsežen pojav spolnih trosišč (peritecijev) glive *Botryosphaeria dothidea* na skorji.

New Disease Reports

First report of *Botryosphaeria dothidea* causing bark dieback of European hop hornbeam in Slovenia

D. Jurc*, N. Ogris, T. Grebenc and H. Kraigher

Slovenian Forestry Institute, Večna pot 2, 1000 Ljubljana, Slovenia

***dusan.jurc@gozdis.si**

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Extensive mortality of European hop hornbeam (*Ostrya carpinifolia*) has occurred in the western part of Slovenia, where this tree species is invading abandoned grassland. The Slovenian Forest Service first reported damage to hop hornbeam in 1997. The extent and intensity of the phenomenon depends on weather conditions. Thus, in a drought year (2003) the affected area covered 6,800 ha with a 50% mortality of hop hornbeam; while in a wetter year (2004) mortality dropped to 35% with 133 ha affected. Bark necrosis is characteristic of the disease. It can develop in healing cankers or the lesions expand to include the whole trunk or branches of the tree, which consequently die (Fig. 1).

Mass occurrence of *Botryosphaeria dothidea* ascomata in winter and *Fusicoccum aesculi* conidiomata in the growing season have been regularly observed on the dead bark of hop hornbeam. Asci measured 80-98.5 (85.5) × 17-20 (18) µm, ascospores 14-34 (22) × 6-9.5 (8) µm and conidia from culture 20.5-30 (25.5) × 4.5-7 (6) µm (Fig. 2). Samples of conidiomata, ascomata and isolations from dead bark on malt extract agar (MEA) were deposited at the Herbarium of the Slovenian Forestry Institute (Acc. Nos 1508-1510). The pathogen had been previously reported as *Botryosphaeria ribis* (Jurc *et al.*, 2003), but with new delineations in the taxonomy of the pathogen (Slippers *et al.*, 2004) the true identity of the fungus has been unravelled. The sequences of the rDNA ITS region from pure cultures (GenBank accession numbers AJ938004 & AJ938005) share 99-100 % similarity with several deposited sequences of *B. dothidea* isolates, hence indicating a clear identification.

Inoculations of two isolates taken from the necrotic bark of hop hornbeam and isolated on MEA were performed in nature in six replications on hop hornbeam coppice shoots. The bark was removed with a cork borer (diameter 6 mm) and an agar plug was inserted (with mycelium facing towards the stem). The wound was covered with micropore tape. Control stems were inoculated with sterile agar plugs. After one year the average necrosis of inoculated stems was 6.7 cm long, while the controls had healed (Fig. 3). A *Fusicoccum* anamorph was reisolated from the edge of the necrosis thus fulfilling Koch's postulates.

B. dothidea has a world-wide distribution and is capable of infecting numerous plant species. Its host range comprises mostly trees and shrubs and even 70 years ago it was reported from 68 genera (Smith, 1934); the host range was later increased by an additional 17 genera (Hepting 1971). In North America it occurs on *Ostrya virginiana* with saprobic activity (Hepting 1971). It has been found on a fallen *O. carpinifolia* branch in Europe with no indication of its pathogenicity (Slippers *et al.*, 2004). This is the first report of *Botryosphaeria dothidea* causing dieback of *Ostrya carpinifolia* in epiphytotic dimensions in Slovenia.

Figure 1: A. dead shoots of hop hornbeam, B. healing canker, C. necrosis of bark with abundant formation of conidiomata

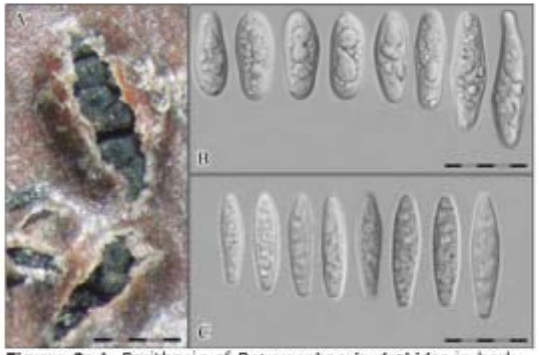


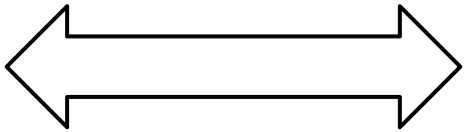
Figure 2: A. Perithecia of *Botryosphaeria dothidea* in bark of *Ostrya carpinifolia* (bar 0,5 mm), B. ascospores (bar 20 Âµm), C. conidia from culture (bar 20 Âµm)

Botryosphaeriaceae

- družina gliv Botryosphaeriaceae je prisotna na različnih drevesnih vrstah
- poznamo jih kot endofite oz. kot latentne agresivne patogene, ki običajno povzročajo bolezni na oslabljenih rastlinah
- družina Botryosphaeriaceae je taksonomsko precej zapletena
  - → morfološka podobnost gliv iz te družine otežuje določanje do nivoja vrste
  - → molekularne metode so omogočile ponovno ovrednotenje taksonomije te družine
  - → z molekularnimi metodami lahko pravilneje določimo in uvrstimo glive v sistem

Endofitne glive = glive, ki celoten ali le del življenjskega kroga, živijo znotraj rastlinskih tkiv in ne povzročajo bolezenskih simptomov.

Okoljski dejavniki
Starost



Rastlina-endofit:
Mutualizem
Komenzalizem
Parazitizem
Vloga pri razgradnji

Črni gaber (*Ostrya carpinifolia* Scop.)

- Svetloljubna drevesna vrsta
- Topla južna pobočja
- Dobro prenaša daljša sušna obdobja; pionirska vrsta sušnih rastišč
- Odporen proti boleznim

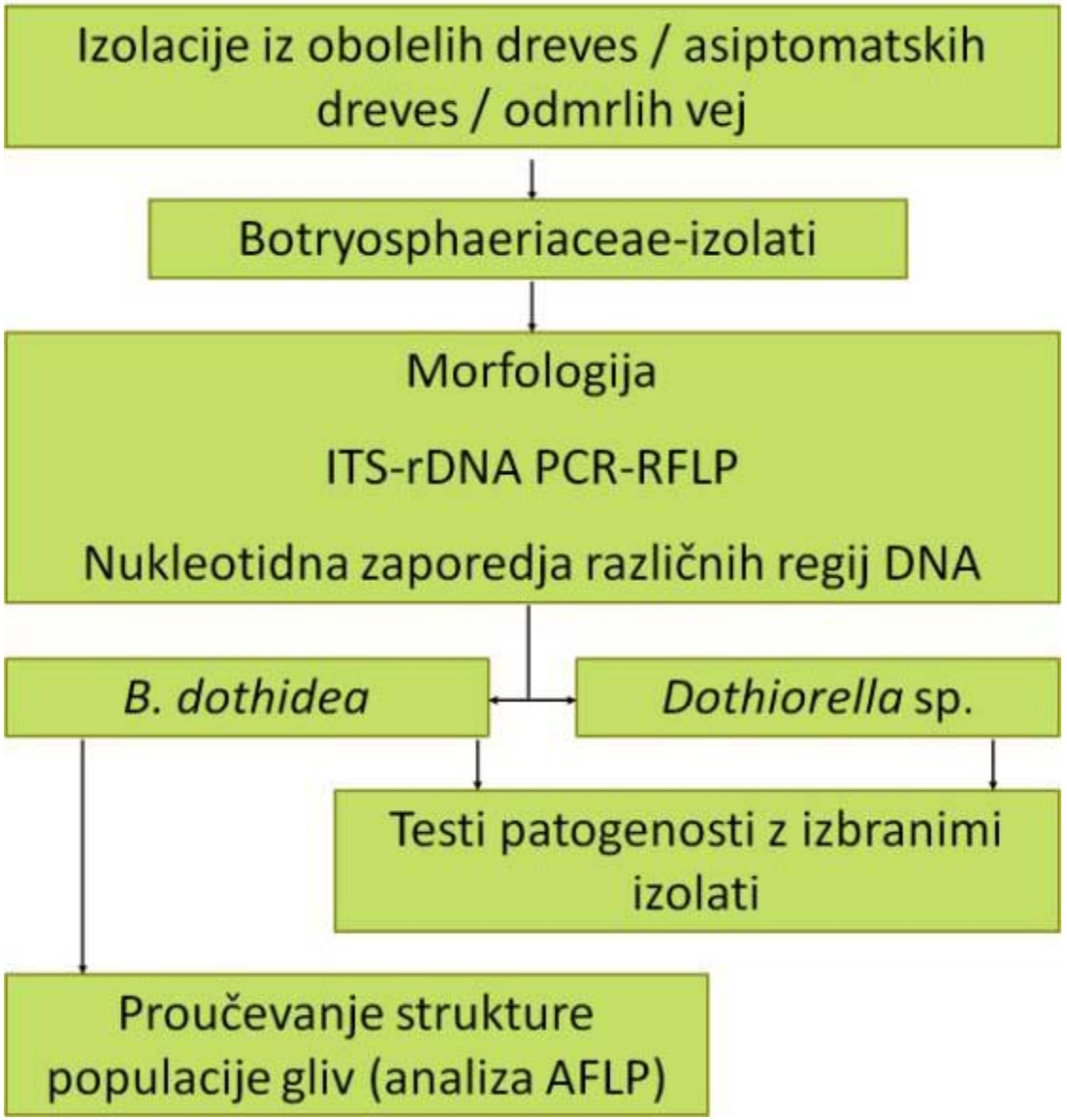
- Gliva *B. dothidea* do l. 1997 ni bila poznana kot povzročiteljica bolezni pri črnih gabrih

1. Kakšna je med- in znotraj-vrstna raznolikost gliv iz družine Botryosphaeriaceae, ki smo jih izolirali iz obolelih črnih gabrov in nekaterih drugih drevesnih vrst v Sloveniji?

2. Ali je gliva *B. dothidea*, ki je bila prepoznana kot primarni vzrok sušenja črnega gabra na Krasu, vnešen invazivni patogen ali pa so neobičajne vremenske razmere povzročile, da je že prisotna glivna populacija prešla iz latentne v aktivno patogeno delovanje?

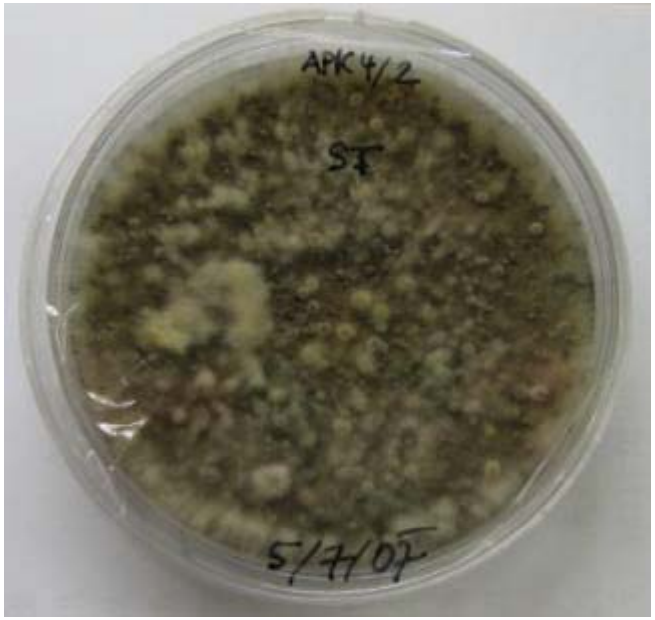
Agricultural Biotechnology Institute (FABI), Faculty of Natural and Agricultural Sciences, University of Pretoria, Pretoria, South Africa

- **Dr. Bernard Slippers**
- **Dr. Draginja Pavlic**


Izolacije iz obolelih dreves / asiptomatskih dreves / odmrlih vej
Botryosphaeriaceae-izolati
Morfologija
ITS-rDNA PCR-RFLP
Nukleotidna zaporedja različnih regij DNA
B. dothidea
Dothiorella sp.
Testi patogenosti z izbranimi izolati
Proučevanje strukture populacije gliv (analiza AFLP)

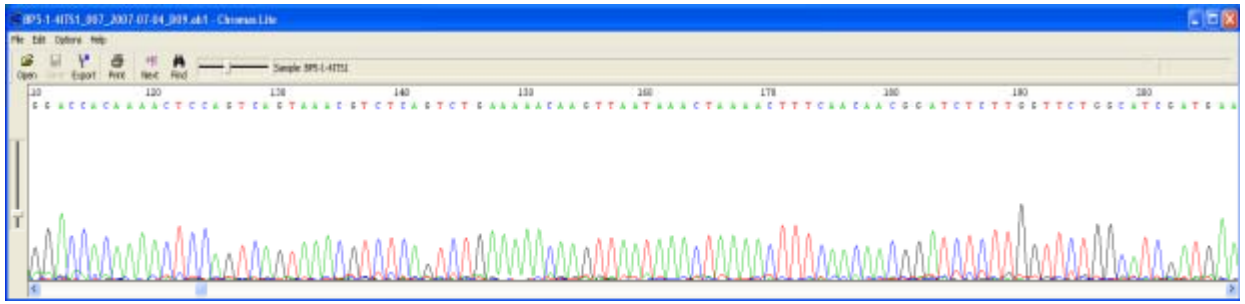
Morfološke značilnosti – *B. dothidea*

- *Fusicoccum*-konidiji
- 24˙1 × 6˙0 µm
- brez sept
- prozorne & vretenaste



Morfološke značilnosti – *Dothiorella sp.*

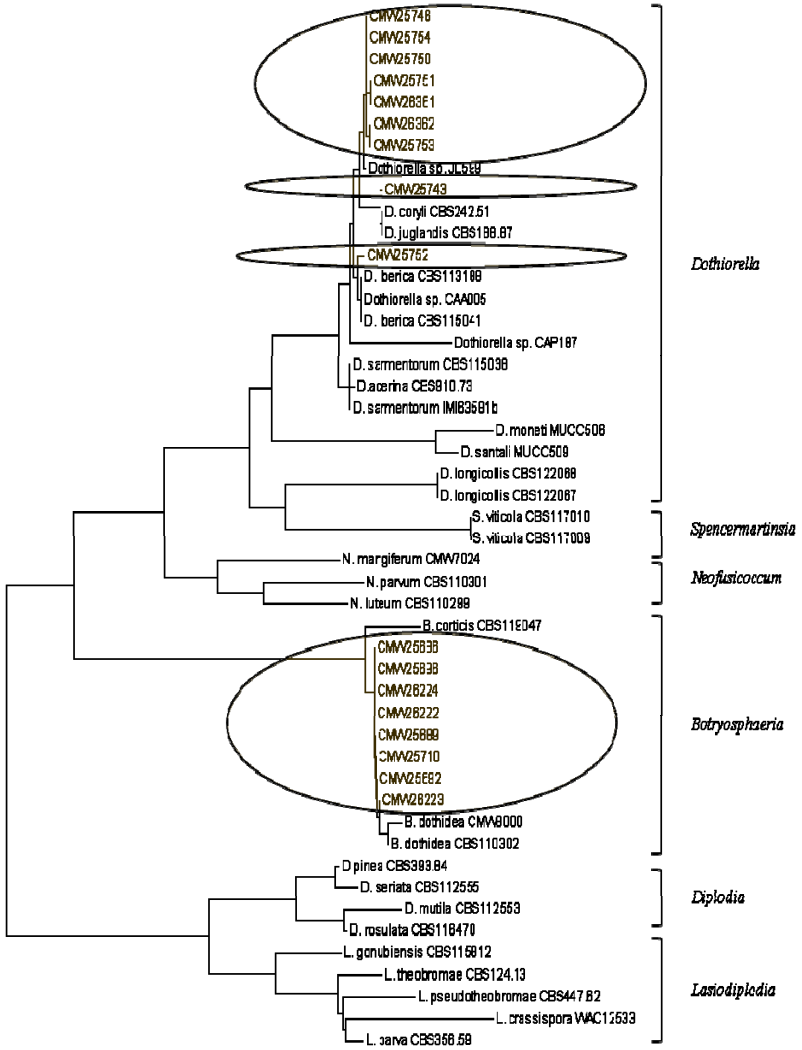
- *Diplodia*-konidiji
- 20˙9 × 9˙8 µm
- 1–3 septe (pregrade)
- pigmentirani



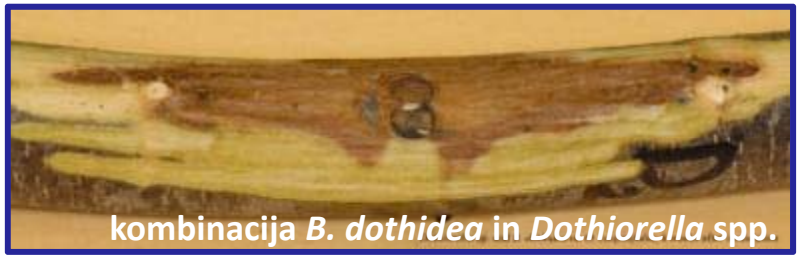
…GACCACAAAACTCCAGTCTGAAAAACAACGG…

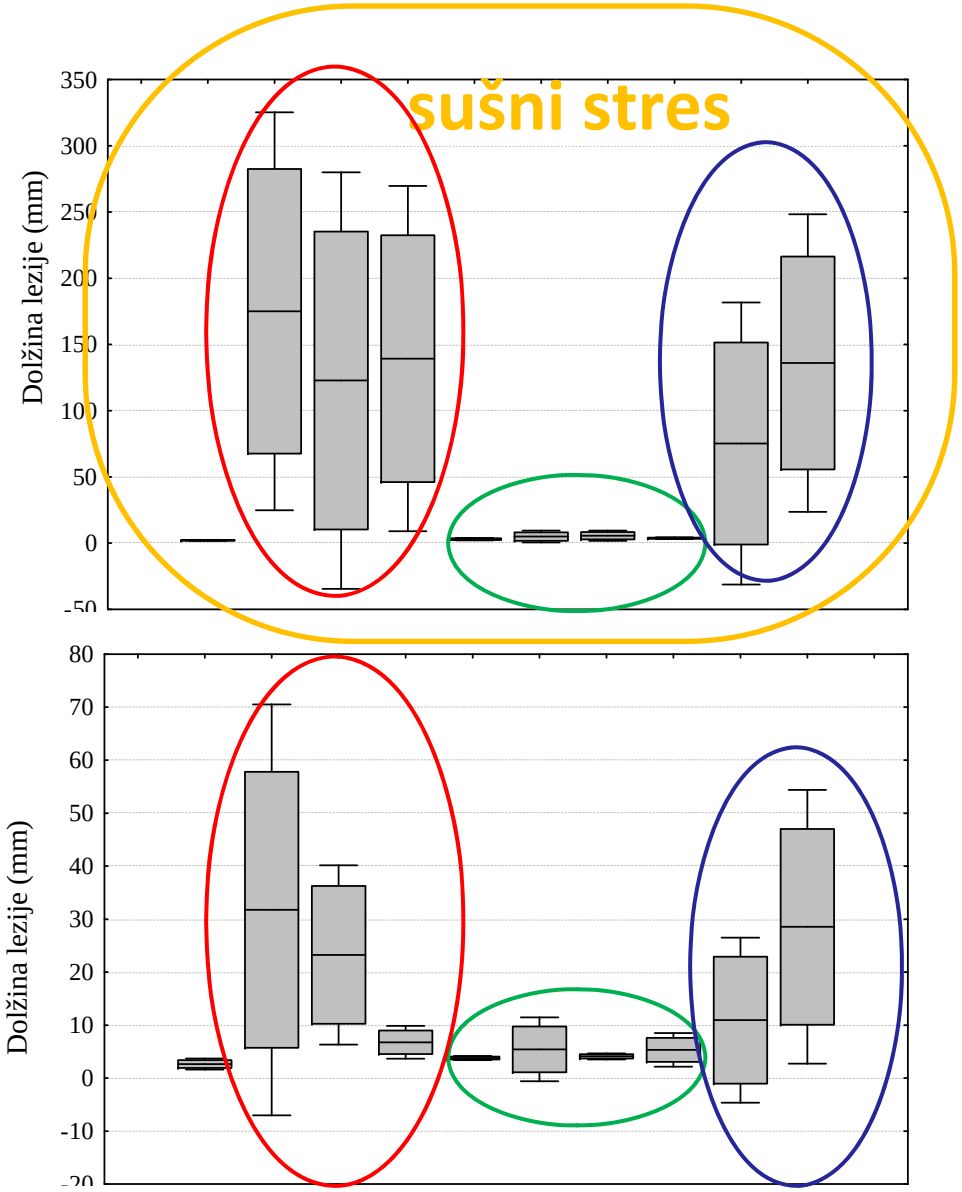
Filogenetsko drevo

- NJ
- kombinirani podatki EF-1α in ITS



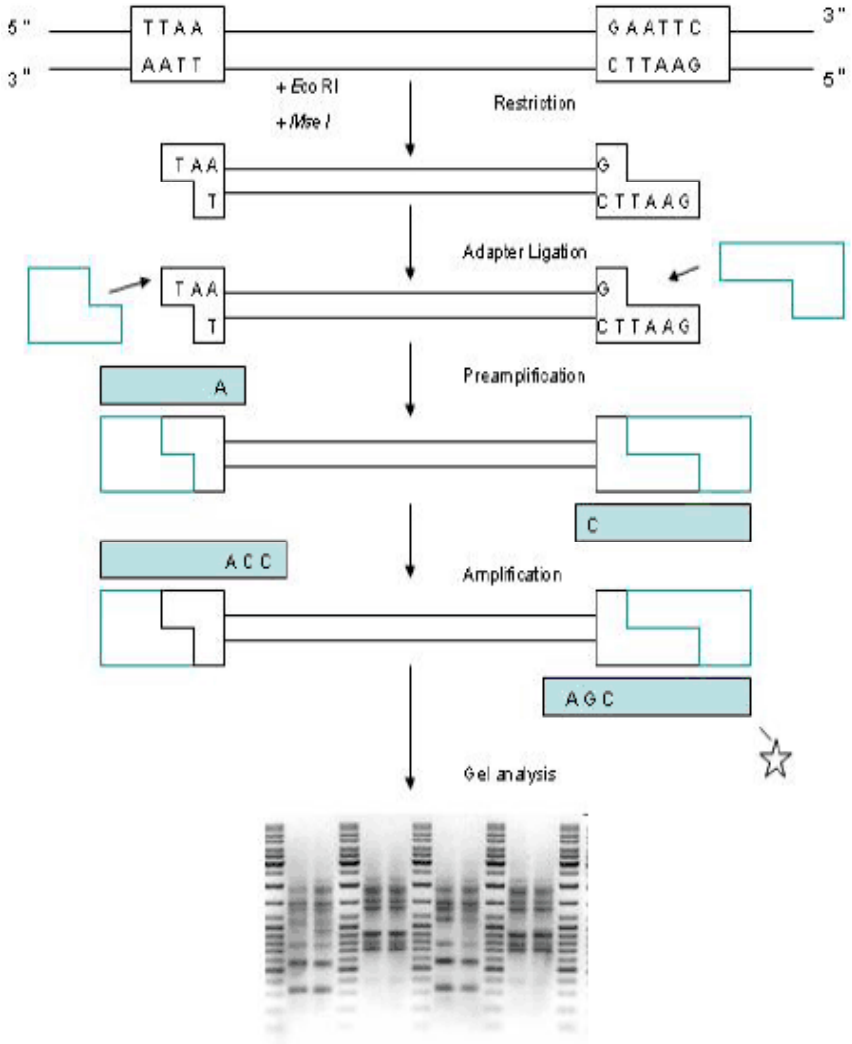
Test patogenosti





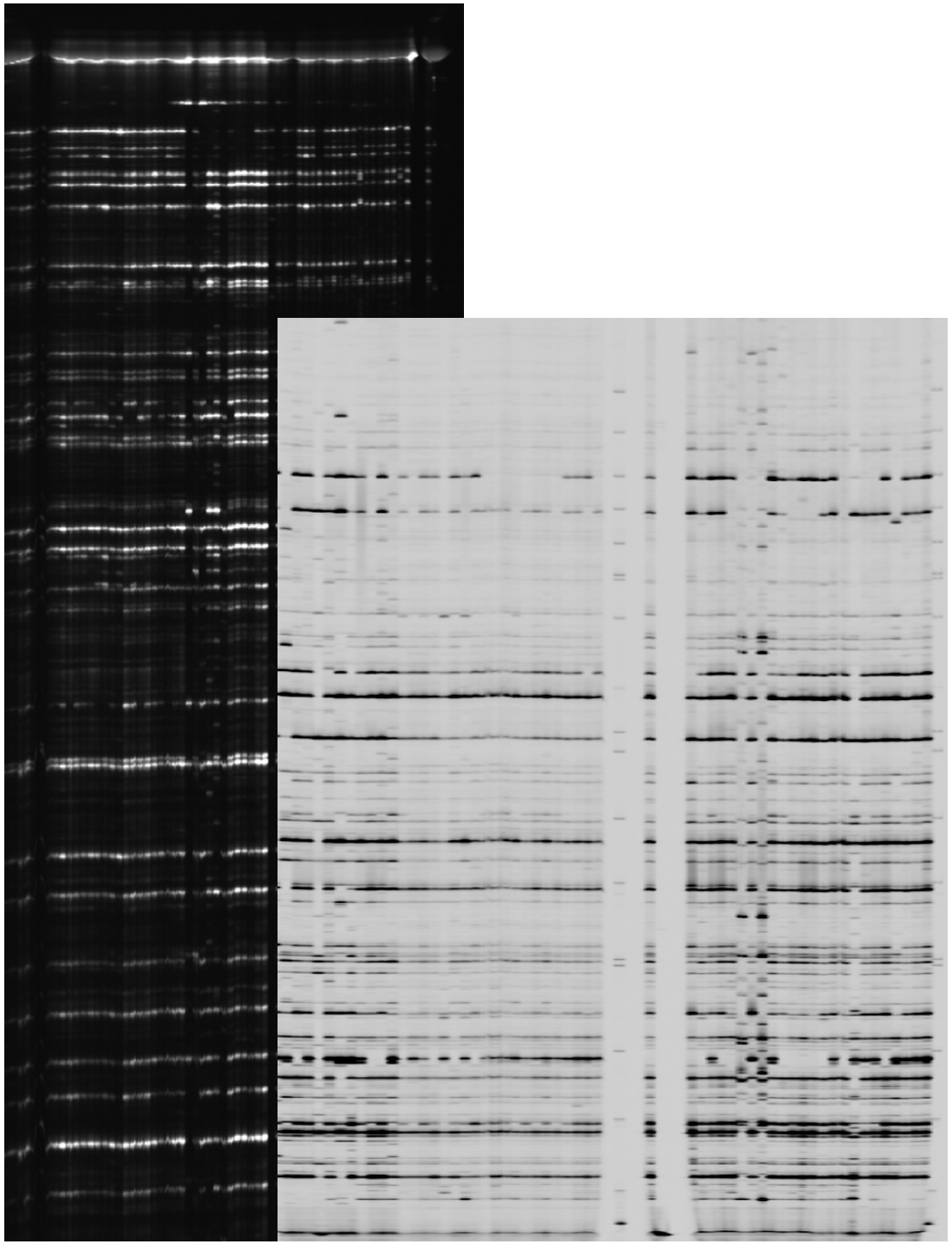
AFLP = dolžinski polimorfizem namnoženih fragmentov

(ang. Amplified Fragment Length Polymorphism)



- molekularna metoda
- detekcija večjega števila polimorfizmov DNA – ***PRSTNI ODTISI***
- analiza celega genoma
- ovrednotenje: prisotnost / odsotnost fragmenta
- primerna metoda za ugotavljanje razlik med sorodnimi organizmi (npr. znotrajvrstna variabilnost)

AFLP



AFLP = dolžinski polimorfizem namnoženih fragmentov
(ang. Amplified Fragment Length Polymorphism)

- razpršenost izolatov *B. dothidea* v AFLP-dendrogramu
- korelacija z mestom izolacije (asimptomatsko/simptomatsko drevo) in geografsko lokacijo ni razvidna
- izolati izolirani iz ostrolistnega javorja in ruja
- AFLP-markerji → heterogena (raznolika) populacija *B. dothidea*

➢ *B. dothidea*

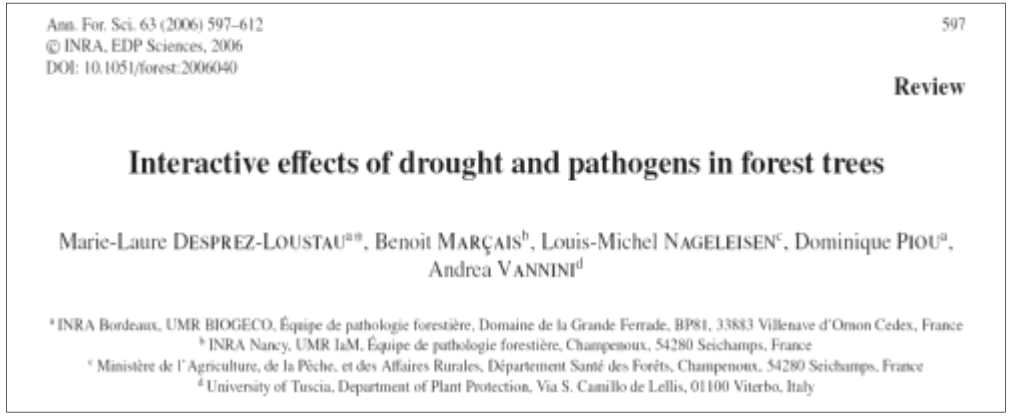
- najpogostejša glivna vrsta, izolirana iz obolelih črnih gabrov v Sloveniji
- statistično značilna patogenost v testih patogenosti
- najverjetneje ključna pri razvoju bolezni
- raznolikost populacije na prizadetem območju

➢ *Dothiorella* spp.

- izolirali smo tri različne skupine gliv iz rodu *Dothiorella*, najmanj ena je verjetno nova vrsta
- patogenost v testih patogenosti NI statistično značilna
- najverjetneje te glive nimajo bistvenega vpliva na razvoj bolezni

- Prisotni latentni patogeni iz družine Botryosphaeriaceae, npr. *B. dothidea*, so pomembni pri razvoju bolezni, ki se je pojavljala na črnih gabrih v Sloveniji v 90-letih prejšnjega stoletja.

- Razširjanje in intenziteta fenomena sušenja črnih gabrov sta bila vzporedna z vremenskimi spremembami (vročina, suše), ki imajo verjeten in pomemben vpliv na razvoj bolezni rastlin.



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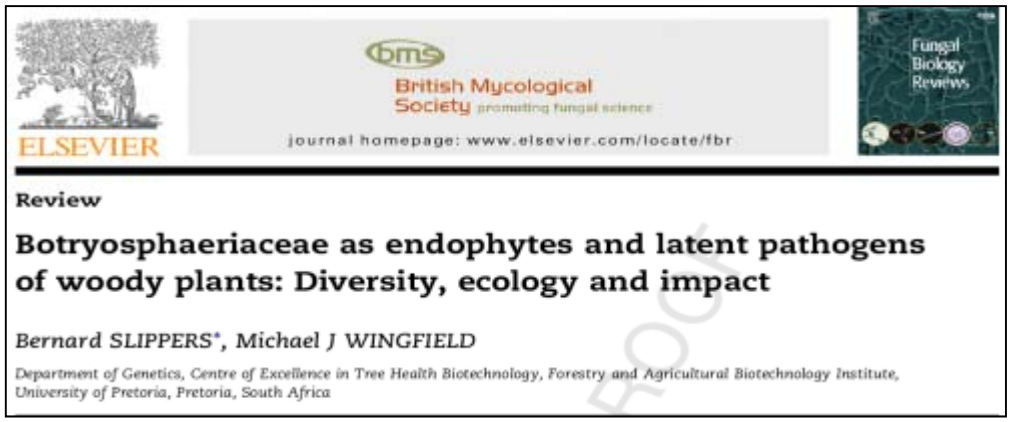
Review

Interactive effects of drought and pathogens in forest trees

Marie-Laure DESPREZ-LOUSTAU[a*], Benoit MARÇAIS[b], Louis-Michel NAGELEISEN[c], Dominique PIOU[a], Andrea VANNINI[d]

[a] INRA Bordeaux, UMR BIOGECO, Équipe de pathologie forestière, Domaine de la Grande Ferrade, BP81, 33883 Villenave d'Ornon Cedex, France
[b] INRA Nancy, UMR IaM, Équipe de pathologie forestière, Champenoux, 54280 Seichamps, France
[c] Ministère de l'Agriculture, de la Pêche, et des Affaires Rurales, Département Santé des Forêts, Champenoux, 54280 Seichamps, France
[d] University of Tuscia, Department of Plant Protection, Via S. Camillo de Lellis, 01100 Viterbo, Italy



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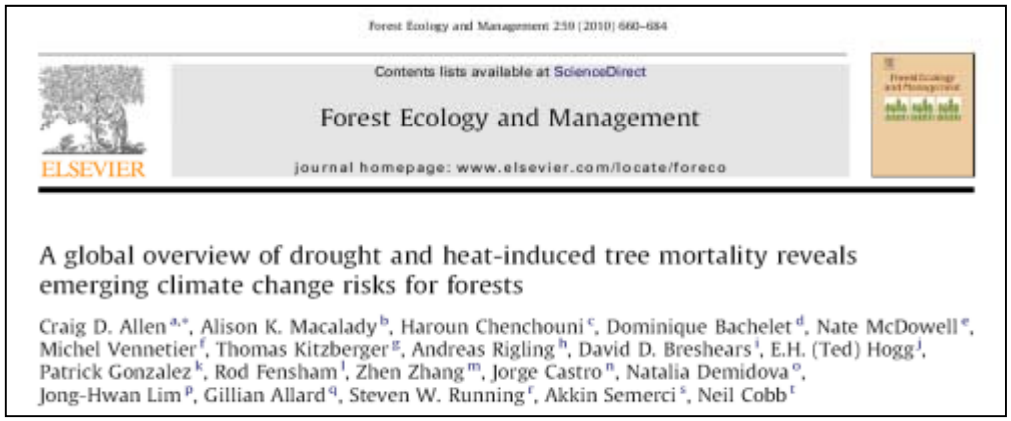
Fungal Biology Reviews

Review

Botryosphaeriaceae as endophytes and latent pathogens of woody plants: Diversity, ecology and impact

Bernard SLIPPERS, Michael J WINGFIELD*

Department of Genetics, Centre of Excellence in Tree Health Biotechnology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, South Africa



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A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests

Craig D. Allen[a,*], Alison K. Macalady[b], Haroun Chenchouni[c], Dominique Bachelet[d], Nate McDowell[e], Michel Vennetier[f], Thomas Kitzberger[g], Andreas Rigling[h], David D. Breshears[i], E.H. (Ted) Hogg[j], Patrick Gonzalez[k], Rod Fensham[l], Zhen Zhang[m], Jorge Castro[n], Natalia Demidova[o], Jong-Hwan Lim[p], Gillian Allard[q], Steven W. Running[r], Akkin Semerci[s], Neil Cobb[t]

Predvidevanja, da bodo klimatske spremembe:

- močno vplivale na latentne oportunistične patogene
- povečale pojavnost novih gozdu škodljivih organizmov.

Diversity and pathogenicity of Botryosphaeriaceae on declining *Ostrya carpinifolia* in Slovenia and Italy following extreme weather conditions

B. Piškur, D. Pavlic, B. Slippers, N. Ogris, G. Maresi, M. J. Wingfield and D. Jurc

Oddelek za varstvo gozdov, Gozdarski inštitut Slovenije, Slovenija
Forestry and Agricultural Biotechnology Institute (FABI), Pretoria, Južna Afrika
IASMA Research Center, San Michele all'Adige, Italija