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UDK: 630*443

Оригинални научни рад

**PHENOTYPIC, ITS PHYLOGENETIC AND PATHOGENIC
ANALYSES OF *PHYLLOSTICTA MULTICORNICULATA*
ASSOCIATED WITH NEEDLE BLIGHT SYMPTOMS OF
BALSAM FIR IN CANADA**

Abstract: *Phyllosticta multicorniculata* Bissett & Palm causes needle blight on *Abies balsamea* (L.) Mill. in eastern Canada. The disease symptoms are similar to those provoked by *Phyllosticta abietis* Bissett & Palm on *Abies grandis* Lindl. in the U.S.A. Preliminary study, based on rDNA ITS sequence data analysis, revealed that *P. multicorniculata* formed distinct but closely-related clade with other sister mitosporic *Phyllosticta* and teleomorphic *Guignardia* and *Botryosphaeria* taxa. However, the phylogenetic position of the fungus is still doubtful which demand further and more advanced molecular investigation. This is the first report of a microconidial stage in *P. multicorniculata* and, for the first time, description of the developing mucilaginous appendages in macroconidia. This study contributes in disease diagnostics and gives new information on distribution of the disease in Canada.

Key words: *Phyllosticta multicorniculata*, Balsam fir, needle blight

**ФЕНОТИПСКЕ, ITS - ФИЛОГЕНЕТСКЕ И ПАТОГЕНЕ АНАЛИЗЕ
PHYLLOSTICTA MULTICORNICULATA У ВЕЗИ СА СИМПТОМИМА
ПРОПАДАЊА ЧЕТИНА НА АМЕРИЧКОЈ БАЛЗАМАСТОЈ ЈЕЛИ**

Извод: У источном делу Канаде забележена је гљива *Phyllosticta multicorniculata* Bissett & Palm, као важан узрочник опадања иглица и антракнозе код *Abies balsamea* (L.) Mill. Симптоми обољења су слични онима изазваним од *Phyllosticta abietis* Bissett & Palm на *Abies grandis* Lindl. у Сједињеним Америчким Државама. Базирајући се на прелиминарним ITS rDNK анализама из ове студије, може се рећи да је гљива сродна врстама из рода *Phyllosticta* spp. (несавршени облик) и неким врстама савршеног облика *Guignardia* и *Botryosphaeria*. Па ипак, није било могуће утврдити тачну филогенетску позицију код *P. multicorniculata*, што подразумева укључивање додатних и детаљнијих молекуларних

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анализа. У раду се износе први налази о постојању микро-конидијског циклуса код ове гљиве и даје детаљан опис гљиве, приказујући карактеристичне етапе у развоју желатинозног привеска код макроконидија. Ова истраживања су спроведена са циљем да побољшају дијагнозу обољења, поткрепљујући налазе новим информацијама које се тичу географског распрострањења овог обољења у Канади.

Кључне речи: *Phyllosticta multicorniculata*, Америчка балзамаста јела, пропадање четина

1. INTRODUCTION

Balsam fir (*Abies balsamea* (L.) Mill.) is economically a very important coniferous tree in eastern Canada. Among numerous needle diseases of Balsam fir, found in the Sherbrooke region of the province of Quebec (Vujanović, St-Arnaud, 2001), one unusual coelomycetous fungus was frequently associated with needle blight symptoms. Usually, the fungal attack causes a reddish colour and needle necrosis resulting in mortality of leading branches and severe crown damage. In order to establish an efficient control strategy, the isolation and identification as well as preliminary molecular and pathogenic analyses of this fungal species were completed in laboratories of the Plant Biology Research Institute/Institut de recherche en biologie végétale (IRBV) located within the Montreal Botanical Garden.

2. MATERIAL AND METHODS

For this study, a collection of plant material was carried out according to a methodology reported by Vujanović & St-Arnaud (2001).

rDNA region amplification was carried out using the ITS1/ITS 4 primer set (White *et al.*, 1990). PCR amplicons were purified for sequencing from agarose gels using the QIAquick gel extraction kit, and sequencing was performed by the Montréal Genomic Centre (Montréal, Qc, Canada). Sequence data were aligned using ClustalW and then imported into PAUP (version 4.0b10) (Swoffors, 2003). The resulting tree was exported to TreeView (Page, 1996) and rooted using the outgroup *Rhexocercosporioides carotae* (DQ526376).

3. RESULTS

Phyllosticta multicorniculata Bissett & Palm was identified to be the only organism associated with needle-blight symptoms in *A. balsamea* (Fig. 1A), occurred during 1999 and 2000 in southern Québec, Canada. This is the first report of this fungal species in the province of Québec. The fungus was isolated on PDA from both healthy and symptomatic needles. From a total of 160 symptomatic needles per year, 62% were colonized in 1999 and 68% in 2000.

Description: All morphological characters of this fungal specimen agrees with Bissett and Palm's (1989) concept of *Phyllosticta* and the *P. multicorniculata* description, in having immersed unilocular and ostiolate pycnidia (Fig. 1B, 1C), and unicellular and multi appendaged mucilaginous conidia (Fig. 1D). In this study, two kinds of conidiogenous cells were found in conidiomata cavity: i) those producing macroconidia, lining the base and most of the side walls and, ii) those producing microconidiogenous cells, lining the ostiolar channel (Fig. 2A). It is important to emphasize that the occurrence of microconidiogenesis in *P. multicorniculata* is an unusual feature for this

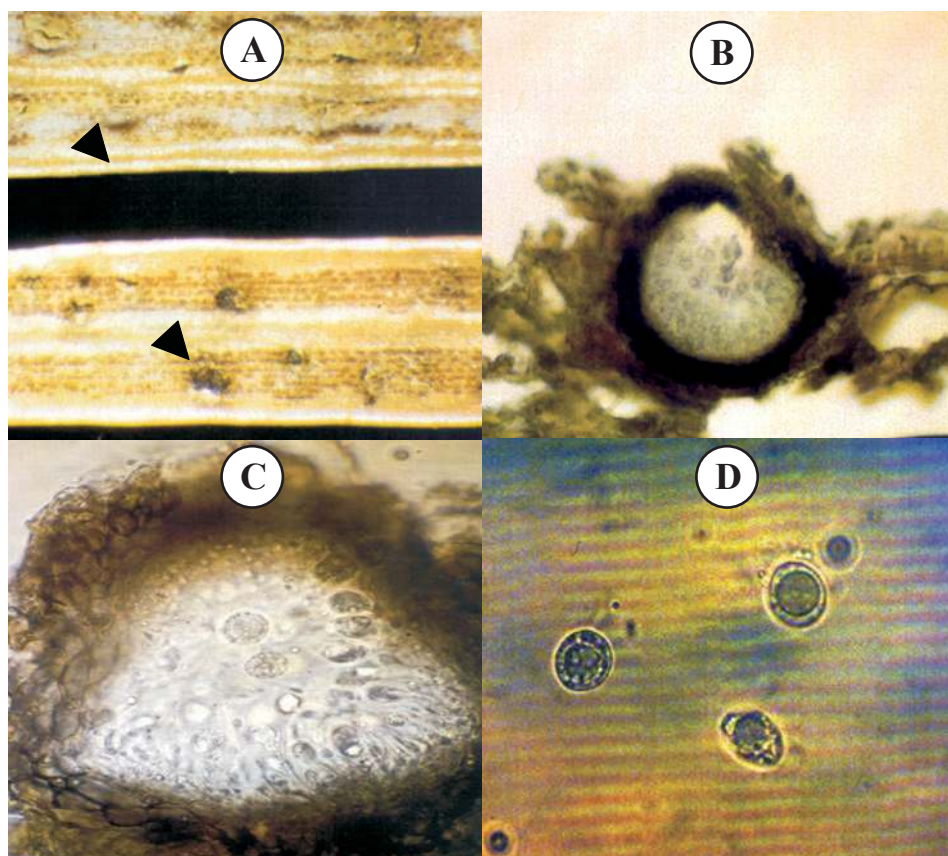


Figure 1. *Phyllosticta multicorniculata* on needles of *Abies balsamea*: **A.** Fructifications on symptomatic needles (arrows), **B.** Pycnidium transversal section, **C.** Enlarged view of pycnidium, **D.** Multi appendaged (arrows) conidia (Vuĵanović: original)

Слика 1. *Phyllosticta multicorniculata* на четинама *Abies balsamea*: **A.** Плодоносна тела гљиве на зараженим четинама (стрелица), **B.** Попречни пресек кроз пикнид, **C.** Увећани пресек пикнида, **D.** Привесци на конидијама (стрелице) (фото: Вујановић В.)

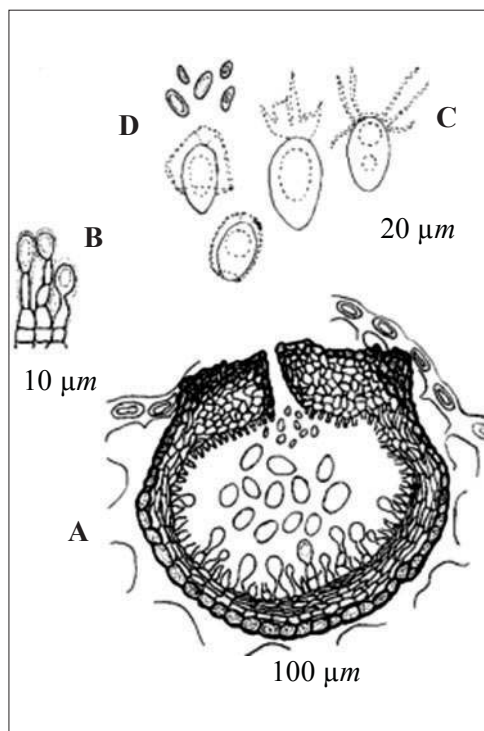


Figure 2. *Phyllosticta multicorniculata*: **A.** Macro and microconidia formation in pycnidium, **B.** Conidiogenesis, **C.** Different stages of developing appendages in macroconidia and, **D.** Microconidia (Vujanović: original)

Слика 2. *Phyllosticta multicorniculata*: **A.** Макро и микро конидије образоване у пикниду, **B.** Конидиогенеза, **C.** Различити степен развојка привезака код макроконидија, **D.** Микроконидије (фото: Вујановић В.)

genus. The macroconidial formation and different stages of appendages' development in macroconidia are shown in Fig. 2B and 2C.

Phylogeny: Parsimony analyses of sequence data showed (Fig. 3) that *P. multicorniculata* isolates belong to distinct but closely-related clades with other sister mitosporic *Phyllosticta* spp. as well as teleomorphic *Guignardia* and *Botryosphaeria* taxa. For more details about taxonomical studies in *Phyllosticta* and their connections with teleomorphs in *Guignardia* and *Discochora*, the work of Van der Aa and Vanev (2002), Van der Aa (1973), Bisset (1990, 1986/a,b), Punithalingam (1974) and Nag Raj (1993) should be consulted.

Culture: On PDA, the fungus produced an olivaceous to greenish black culture with white margin (Fig. 4A). Colonies were of slow growth (approx. 3 cm diam. for 2 weeks at 21°C in darkness) forming dense mycelia. Hyphae were hyaline or slightly coloured, 2-4 µm in diameter, septate and usually forked at the ends (Fig. 4B).

Pathology: In vitro, all three tested *P. multicorniculata* isolates showed pathogenic properties on balsam fir causing tissue necrosis of artificially infected seeds and seedlings (Fig. 4C).

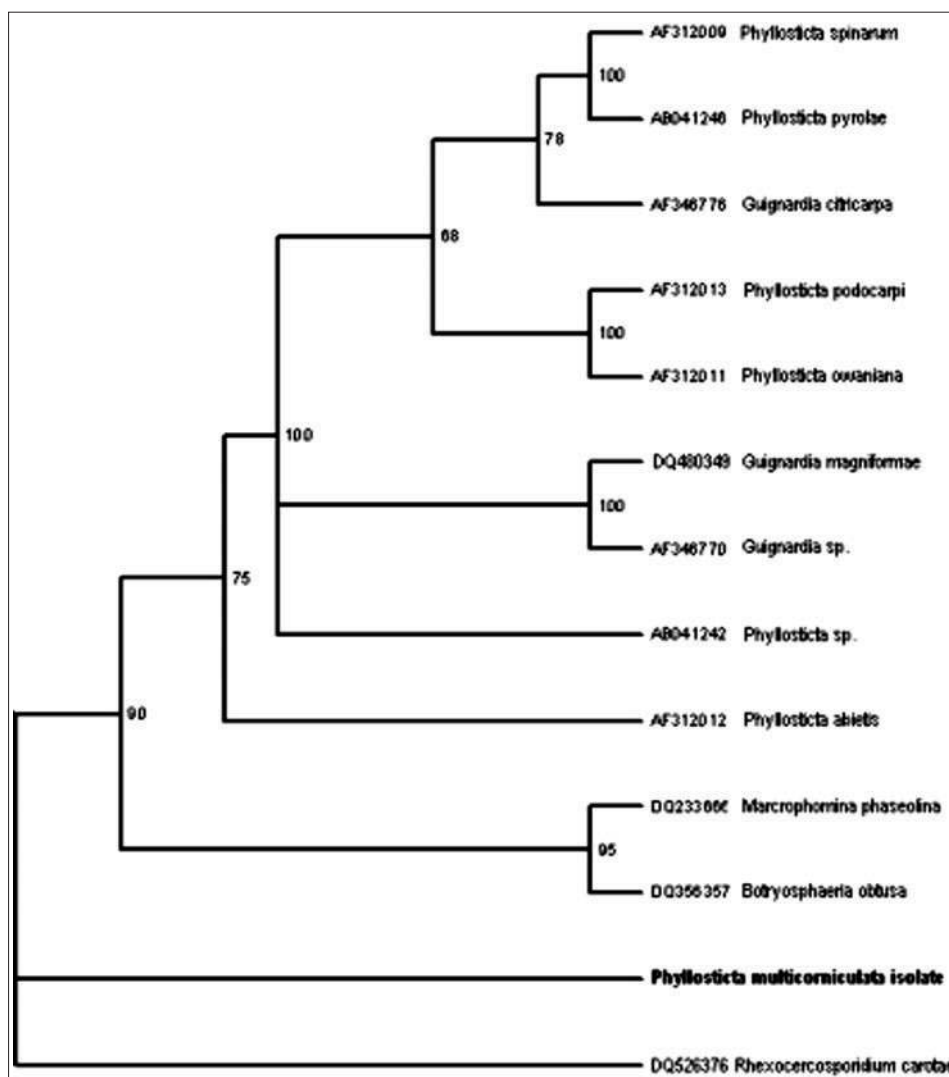


Figure 3. Relationship of *Phyllosticta multicorniculata* and other closely related *Guignardia* and *Botryosphaeria* species based on internal transcriber spacer region rDNA and maximum parsimony analyses. Bootstrap support values greater than 75% for 1000 replicates are shown at the nodes. GenBank accession numbers are given before species names. Nominated outgroup species was *Rhexocerosporioides carotae* (DQ526376)

Слика 3. Однос *Phyllosticta multicorniculata* и других уско сродних *Guignardia* и *Botryosphaeria* врста на основу ITS региона rDNA и анализе максималне очуваности. Вредности Bootstrap-а веће од 75%, за 1.000 понављања, приказане су на местима гранања. Генске базе података приказане су испред врста. Као контролна врста узета је *Rhexocerosporioides carotae* (DQ526376)

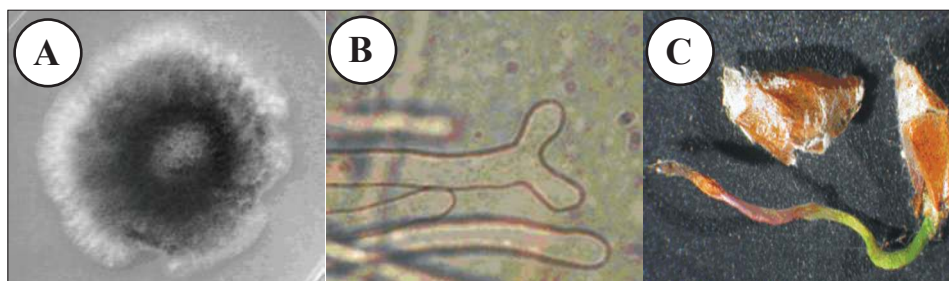


Figure 4. *Phyllosticta multicorniculata*: **A.** 2-weeks old culture on PDA, **B.** fork-like terminal hyphae, **C.** necrotic symptoms on seed and young seedling (Vujanović: original)

Слика 4. *Phyllosticta multicorniculata*: **A.** 2 недеље стара култура на ПДА, **B.** на врху рачвасте хифе, **C.** некротични симптоми на семену и клици (фото: Вујановић)

4. DISCUSSION

In Canada, *P. multicorniculatis* is currently known to exist with limited distribution in Ontario (loc. Muskoka) and Prince Edward Island (loc. Queens), occurring exclusively on the *Abies balsamea* (Fig. 5). The present study clearly indicated that the fungus also occurred on balsam fir in southern Québec (loc. Sherbrooke), 50 km near the Maine, US.

The fungus has been reported previously in US, but only on White fir (*Abies concolor*) in the region of Wisconsin (Wenner, 1997). The real distribution, origin, ecology as well as impact of this fungus on forest ecosystems in Canada are still unknown. This is unfortunate considering a proved pathogenic behaviour of the fungus on seed of balsam fir in vitro as well as mature trees in natural forests.

Phylogenetic position of *P. multicorniculatis* is also still unclear. This preliminary study, based on analyses of ITS rDNA region, showed that the fungus formed distinct clade from *P. abietis*. In addition, the occurrence of microconidiogenesis in *P. multicorniculatis* is also identified. This represents an unusual feature for genus *Phyllosticta*, which needs further taxonomical clarification.

Although, the morphological differences between *P. multicorniculatis* and *P. abietis* are obvious, it is unknown whether these two pathogens could attack and share the same host in a specific geographical region. For example, *Phyllosticta abietis* Bissett & Palm was found on Grand fir (*Abies grandis*) in Idaho (USA) and is considered to be destructive pathogen for Christmas tree plantations (Funk, 1985; Wenner, 1997).

Further taxonomical and ecological studies will be therefore needed in

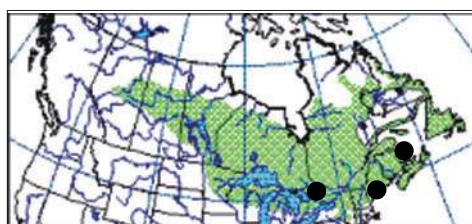


Figure 5. The range of Balsam fir in Canada (● known locations of *Phyllosticta multicorniculata* in Canada)

Слика 5. Распрострањење Америчке балзамсте јеле у Канади (● познате локације *Phyllosticta multicorniculata* у Канади)

order to fill the gap of the knowledge on *Phyllosticta* associated with various *Abies* species in North America. For that purposes, it seems appropriate to conduct a more extensive collection of fungal isolates from *Abies* hosts throughout Canada in order to differentiate *Phyllosticta* species and enlighten their taxonomy, ecology and mycogeography. This data could be use than as useful tools to develop efficient control strategy against these phytopathogenic treats for Canadian boreal forests.

5. CONCLUSION

The study is being published in order to assist diagnostics of disease problems on balsam fir trees and to provide new information for current forest protection efforts in Canadian boreal region. It also supports monitoring, an important asset against exotic pathogens within an international trade of coniferous trees throughout world.

Acknowledgements: We thank the Montreal Botanical Garden for financial support of this research. I am grateful to S.A. Redhead curator of mycological herbarium of Agriculture Canada (DAOM) for the loan of various specimens of *Phyllosticta* [DAOM 1888797 - *P. abietis*; DAOM 114552a - *P. multicorniculata*, DAOM 145406b - *Tiarosporella abietis*] that allowed me to make the necessary comparisons of this species with others of closely related fungal taxa. I also thank J. Muir, Forest Pathologist, BC Ministry of Forests, for the advice to publish these results.

REFERENCES

- Bissett J. (1986/a): *A note on typification of Guignardia*, Mycotaxon 25 (519-522)
- Bissett J. (1986/b): *Discohora yuccae* sp. nov. with *Phyllosticta* and *Leptodothiorelle synanamorpha*, Can. J. Bot. 64 (1720-1726)
- Bissett J. (1990): *Phyllosticta multicorniculata*, Fungi Canadenses 322 (1-2)
- Bissett J., Palm M.E. (1989): *Species of Phyllosticta on conifers*, Can. J. Bot. 67 (3378-3385)
- Funk A. (1985): *Foliar fungi of western trees*, Can. For. Serv. Pac. For. Res. Cent. Rep. BC-X 265
- Nag Raj T.R. (1993): *Coelomycetous Anamorphs with Appendage-Bearing Conidia*, Mycologue Publications, Waterloo, Ontario (1101)
- Page R.D.M. (1996): *TreeView: An application to display phylogenetic trees on personal computer*, Computer Applications in the Biosciences 12 (357-358)
- Punithalingam E. (1974): *Studies of Spaeropsidales in culture*, II. Mycol. Pap. 136 (1-63)
- Swofford D.L. (2000): *PAUP (and other methods)*, Phylogenetic analysis using parsimony, version 4.0b10, Sinauer Associates, Sunderland
- Van der Aa H.A., Vanev S. (2002): *A revision of the species described in Phyllosticta*, Edited by A. Aptroot, R.C. Summerbell and G.J. Verkley, Centraalbureau voor Schimmeltcultures, Utrecht (510)
- Van der Aa, HA. (1973): *Studies in Phyllosticta*, I. Stud. Mycol. 5 (1-110)

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- Vujanović V., St-Arnaud M. (2001): *Pathogenic fungal species on Balsam fir in southern Quebec*, Canadian Plant Disease Survey 81 (152-153)
- Wenner N.G., Merrill W. (1997): *Phyllosticta multicorniculata on Abies spp. in the northeastern US*, Plant Dis. 81 (230)
- White T.J., Bruns T., Lee S., Taylor J.W. (1990): *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*, In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds., PCR protocols: a guide to methods and applications, Academic Press, San Diego (315-322)

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**LES ANALYSES PHÉNOLITYPES, PHILOGÉNÉTIQUES ET PATHOGÈNES DE
PHYLLOSTICTA MULTICORNICULATA AU SUJET DES SYMPTÔMES
DE LA DÉGRADATION DES AIQUILLES CHEZ LE SAPIN BAUMIER AU CANADA**

Résumé

Phyllosticta multicorniculata Bissett & Palm est un champignon qui cause la brûlure des aiguilles sur l'*Abies balsamea* (L.) Mill. dans l'Est du Canada. Les symptômes de cette maladie sont similaires à ceux causés par le *Phyllosticta abietis* Bissett & Palm sur l'*Abies grandis* Lindl. aux États-Unis. Basé sur des analyses préliminaires de la région de l'ITS, le champignon est proche des taxons similaires, qui sont distribués aux trois regroupements: *Phyllosticta* (les anamorphes) et *Guignardia* and *Botryosphaeria* (les téléomorphes). Cependant, la position phylogénétique de *P. multicorniculata* reste non résolue, ce qui demande l'accomplissement d'une étude moléculaire plus poussée. D'ailleurs, c'est la première mention de la présence des microconidies chez *P. multicorniculata* et la première illustration du développement des appendices gélatineuses du macroconidie. Les données présentées représentent une contribution dans le domaine de la diagnostique de la maladie ainsi que de nouvelles connaissances sur la distribution de la maladie au Canada.