Зборник Матице српске за природне науке / Proc. Nat. Sci., Matica Srpska Novi Sad, № 113, 235—241, 2007

UDC 635.82:579.6(497.11+492+497.6)

Jasmina M. Glamočlija[1]*, *Marina D. Soković*[1], *Milica V. Ljaljević-Grbić*[2], *Jelena B. Vukojević*[2], *Ivanka M. Milenković*[2], *L. J. L. D. van Griensven*[3]

[1] Institute for Biological Research "Siniša Stanković", Bulevar despota Stefana 142, Belgrade, Serbia
[2] Institute of Botany, Faculty of Biology, University of Belgrade, Takovska 43, Belgrade, Serbia
[3] Plant Research International, Wagenningen University, The Netherlands
* Author for correspodence

MORPHO-PHYSIOLOGICAL CHARACTERISTICS AND INTERACTIONS OF ISOLATES OF *MYCOGONE PERNICIOSA* (MAGNUS) DELACR.

ABSTRACT: *Mycogone perniciosa* (Magnus) Delacr., which causes wet bubble disease of *Agaricus bisporus* Lange (Imb), results in a considerable crop loss on mushroom farms in Serbia. The isolation and identification of five isolates of *M. perniciosa* from diseased fruit bodies of white button mushroom from mushroom units in Serbia, Bosnia and Herzegovina and Holland were made. Morpho-physiological characteristics and inter-relationships of the obtained isolates were studied. Macroscopic and microscopic investigations of different zones between colonies of the isolates of *M. perniciosa* revealed the phenomenon of the hyphal interference between different isolates. The obtained results suggest that hyphal interference could serve as an additional parametar for a more reliable determination of fungal specifity.

KEY WORDS: *Mycogone perniciosa*, mycopathogen, hyphal interference

INTRODUCTION

Wet Bubble Disease, caused by mycopathogen *Mycogone perniciosa* (Magnus) Delacr., is still considered as one of the most important diseases of the cultivated mushrooms *Agaricus bisporus* Lange (Imb), wherever white button mushroom are produced commercially (Sisto et al., 1997, Sharma and Kumar 2000, Bora and Özaktan 2000, Nanagulyan and Yesayan 2002). Mushroom cultivation in Serbia is still less developed than in other countries, and *M. perniciosa* has a significant influence on quality and yield of mushrooms. *Mycogone perniciosa* produces small thin-walled phialoconidia on *Verticillium*-like conidiophores, together with much larger bicellular

conidia (aleuriospores) that develop on short, lateral hyphae, consisting of dark, spherical thick-walled, verrucose apical cell and thin-walled basal cell. Besides vegetative mycelium, both phialospores and aleuriospores of *M. perniciosa* are infectious (H o l l a n d and C o o k e 1990). Studies of the *M. perniciosa* isolates directed toward the distinction of different isolates, is of great importance from both theorethical and practical point of view. This fungi is known by extremly high mycopathogenic potencial and frequently attacks crops of white button mushroom, a common edible mushroom with major economic value and a cosmopolitan distribution (K e r r i g a n, 1995).

According to W e b s t e r (1970) hyphal interference can be of great significance if the hypotheses that it represents the most obvious and most clear form of interspecific competition, and that it could serve as an additional parametar for determination of different isolates of *M. perniciosa*, are true.

In this study, we examined the morpho-physiological characteristics and inter-relationships of the obtained *M. perniciosa* isolates.

MATERIALS AND METHODS

Samples of diseased mushrooms were collected from mushroom farms in Serbia, Bosnia and Hercegovina and Holland. Pure culture of the *M. perniciosa* was isolated from *A. bisporus* in the Mycological Laboratory, Institute for Biological Research "Siniša Stanković". Fungal isolates used in this work were as follows: MPS from mushroom farms in Bosnia and Herzegovina (Sarajevo); 2 from mushroom farms in Serbia: MPPS (Padinska Skela) and MPR (Ripanj) and 2 (MPH1 and MPH2) from the Mushroom Experimental Station, Wageningen, Holland. The isolates were maintained on potato dextrose agar (PDA). The cultures were stored at 4°C and subcultured once a month (B o o t h 1971).

Morpho-physiological characteristics of the isolates were recorded on the colony, grown on Petri plates with PDA medium, during 10 days, at 25°C. Colony characteristics and growth measurements were made daily. Hyphae, phialoconidia and aleuriospores were placed on microscopic slides and stained with Lactophenol cotton blue (J o h a n s e n, 1940). Measurements (at least 30 of each spore type) and photographs were made on Reichert microscope with Canon power Shot S40.

Different combinations each consisting of three fungal isolates, were inoculated in Petri plates containing PDA medium. The three isolates were inoculated equi-distant from the others in all possible combinations in the same Petri plates. The cultures grown under laboratory conditions (day light, at 25°C) were examined after 5 and 10 days of age.

RESULTS AND DISCUSSION

Colony characteristics of all isolates varied (Table 1). The colonies were very regular in growth with either dense aerial mycelium MPH2, MPPS and

MPR, or sparse MPH1. Only MPS isolate had colony, which grew in sectors compact and aerial, irregular in growth. Colony colour, which to some extent indicates the production of aleuriospores, varied from white MPS to dark brown MPPS (Table 1).

Tab. 1 — Some morphological characteristics of isolates of *Mycogone perniciosa*

Isolates	Colony tipe	Colony size (mm)	Hyphae max-min mostly (µm)	Phyalospores max-min mostly (µm)	Aleuriospores Upper cell (max-min, mostly) Lower cell (max-min mostly) (µm)
MPS	white, growth in sectors, compact and aerial mycelium, irregular growth	75x76x77	4—8 6	2x6—6x18 2x12	12x16—22x24 (18x20) 6x10—10x18 (10x14)
MPPS	dark brown, with white edge, aerial mycelium	68x69x68	3,75—5 3,75	3,75x12,50 — 5x18,75 3,75x12,50	15x17,50—21,25x23,75 (18,75x20) 7,50x11,25—13,75x15 (10x13,75)
MPR	light brown, with white edge, aerial mycelium	70x50x76	2,50—5 3,75	3,75x7,50 — 3,75x11,25 3,75x12,50	12,50x17,50—23,75x25 (18,75x21,25) 7,50x10—12,50x13,75 (12,50x12,50)
MPH1	white, supstrate mycelium	74x70x74	2,50—5 3,75	2,50x7,50 — 2,50x13,75 2,50x11,25	12,50x13,75—22,50x22,50 (20x20) 6,25x6,25—15x17,50 (10x10)
MPH2	amber brown, with white edge, aerial mycelium	80x80x79	2,50—5 3,75	2,50x7,50 — 3,75x14 3,75x12,50	15x17,50—22x22 (18,75x20) 6,25x8,75—12,5x17,50 (8,25x11,25)

Growth rates and sizes of the colonies of all isolates were similar (Table 1). All isolates produced both phialospores and aleuriospores. The most intensive sporulation was detected in MPPS and MPR, then in MPH2, and the lowest intensity was in MPS and MPH1. Phialospores size varied within the range 2x12 µm (MPS) — 5x18,75 µm (MPPS). Aleuriospores varied within the range 23,75 x 25 µm (MPR) to 12,50 x 13,75 µm (MPH1) for the upper cell, and 10 x 18 µm (MPS) to 6,25 x 6,25 µm (MPH1) for the lower cell (Table 1). Our isolates of *M. perniciosa* came from mushroom farms from Serbia, Bosnia and Herzegovina and Holland but differences between them were not so obvious when compared to the other literature data (Gray and Morgan-Jones, 1980; Umar et al., 2000; Sharma and Kumar, 2000 and Potočnik, 2006). In contrast to the data of Smith (1924), Atkins and La Touche (1948), Hsu and Han (1981) and Fletcher et al. (1995) which reported two-cell phialospores, we could not find this form in our isolates.

Albouy and Lapierre (1972) and Fletcher et al. (1995) found that some pathogenic strains of *M. perniciosa*, which were slow-growing on agar, were highly pigmented and produced numerous aleuriospores. Other

were weakly pathogenic, producing much vegetative growth and little pigmentation. The slow-growing forms were found to contain numerous virus like particles.

We observed interactions between all tested isolates after 10 days. There were several types of interaction: detaining of growth at the site of contact without visible changes, overgrowth of mycelium of one isolate and demarcation lines between the isolates (Table 2). According to the morpho-physiological characteristics and hyphal interferences of five *M. perniciosa* isolates, the isolates from Serbia were similar; the isolates from Holand showed mutually similar characteristics, but they were different from the isolates from Serbia. Isolate from Bosnia and Herzegovina was different from these two groups (Figure 1—4).

Tab. 2 — Interactions appearing between colonies of isolates of *Mycogone perniciosa*

Izolat	**MPH2**	**MPH1**	**MPR**	**MPPS**	**MPS**
MPS	detaining of growth at the site of contact	overgrowth of mycelium	demarcation line 4—5 mm	demarcation line	detaining of growth at the site of contact
MPPS	detaining of growth at the site of contact	detaining of growth at the site of contact	demarcation line	demarcation line	
MPR	detaining of growth at the site of contact	overgrowth of mycelium	detaining of growth at the site of contact		
MPH1	overgrowth of mycelium	overgrowth of mycelium			
MPH2	detaining of growth at the site of contact				

Earlier investigations of hyphal interference phenomenon on other fungal species showed different interspecies interaction. As demonstrated previously (Franić-Mihajlović et al., 1996), the isolates of *Diaporthe/Phomopsis* known for their extremely phytopathogenic potential, showed different reactions during the investigation of hyphal interference. Demarcation lines were formed between the isolates which originated from one plant. Formation of the lines between the colonies of the same morphological group of isolates was named as inter-species antagonism by Brayford (1990 a, b). Phenomenon of demarcation line formation results from the incompatibility between genetically different colonies. On the basis of comparison of interactions between the different isolates *M. perniciosa*, we can conclude that these belong to different groups. Genetical difference between these isolates based on molecular-genetic characteristics (Glamočlija, 2006), supports such a conclusion.

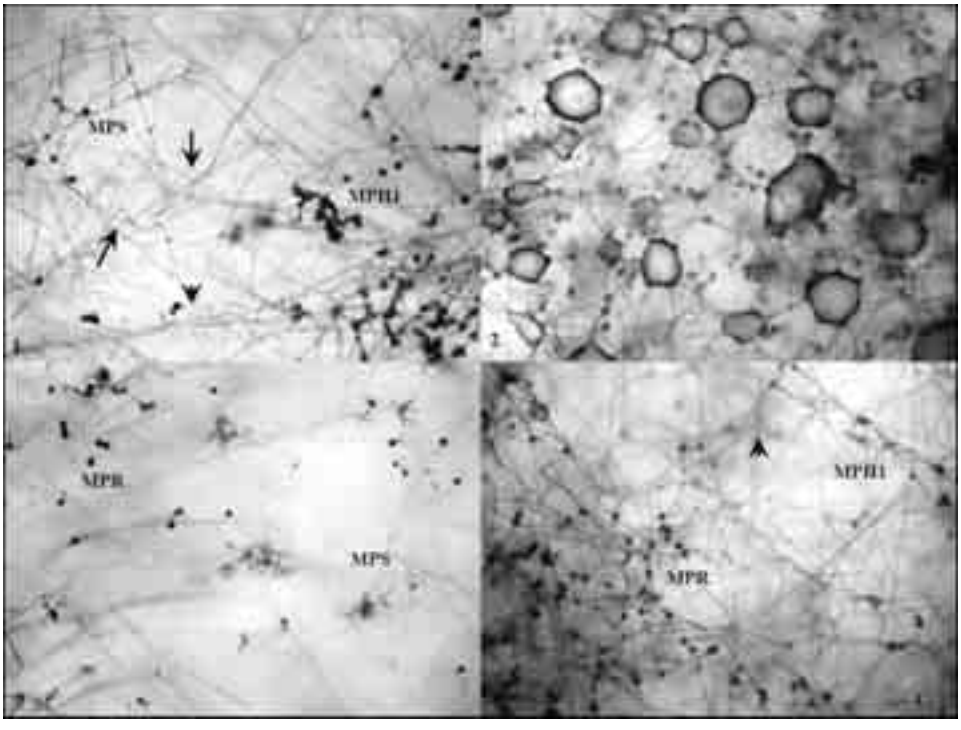

Fig. 1—4 — Types of interactions between isolates of *M. perniciosa*: 1. anastomosis (arrows) of hyphae MPH1 and MPS; 2. overgrowth of MPH1 and MPH2 with exudation; 3. demarcation line between MPR and MPS; 4. anastomosis of hyphae MPH1 and MPR

ACKNOWLEDGMENTS

This study was supported by the Ministry of Science and Environmental protection, Grant# 143041.

REFERENCES

Albouy, J., Lapierre, H. (1972): *Observation en microscopie electronique de souches viruses de Mycogone perniciosa (Magn) agent d'une mole du champignons de couche*, Ann. De Phytopathologie 4: 353—358.

Atkins, F. C., La Touche, C. J. (1948): *Diseases caused by Mycogone perniciosa Magnus*, Mushroom dis. Leafl. 3: 3 pp.

Booth, C. (1971): *Fungal Culture Media*. In: *Methods in Microbiology*, 4, Ed., J. R. Norris, D. W. Ribbons, Academic Press, London & New York, 49—94.

Bora T., Őzaktan, H. (2000): *Biologocal control of some important mushroom diseases in Turkey by fluorescent Pseudomonas*. In: *Mushroom Science XV. Science and Cultivation of Edible Fungi*, Ed. L. J. L. D. Van Griensven, Balkema, Rotterdam, 689—693.

Brayford, D. (1990 a): *Variation in Phomopsis isolates from Ulmus sp. In the British Isles and Italy*, Mycol. Res., 94: 691—697.
Brayford, D. (1990 b): *Vegetative incompatibility in Phomopsis from elm*, Mycol. Res., 94: 745—752.
Fletcher, J. T., Jaffe, B., Muthumeenakshi, S., Brown, A. E., White, D. M. (1995): *Variations in isolates of Mycogone perniciosa and in disease symptoms in Agaricus bisporus*, Plant Pathology 44: 130—140.
Franić-Mihajlović, D., Vukojević, J., Mitić, N. (1996): *Hyphal interference of Diaporthe/Phomopsis Helianthi with related species*, Helia 19 (24): 79—86.
Glamočlija, J. (2006): *Prevencija i tretman Mycogone perniciosa Magn. u gajilištima Agaricus bisporus (J. Lange) Imbach*, doktorska disertacija, Biološki fakultet, Univerzitet u Beogradu.
Gray, D. J., Morgan-Jones, G. (1980): *Notes on Hyphomycetes 34: some mycoparasitic species*, Mycotaxon 10: 374—404.
Holland, D. M., Cooke, R. C. (1990): *Activation of dormant conidia of the wet bubble pathogen Mycogone perniciosa by Basidiomycotina*, Mycological Research 94: 789—792.
Hsu, H. K., Han, Y. S. (1981): *Physiological and ecological properties and chemical control of Mycogone perniciosa Mang. Causing wet bubble in cultivated mushrooms*, Agaricus bisporus. Mush. Sci. XI: 403—425.
Johansen, D. A. (1940): *Plant Microtechnique*, Mc Graw-Hill Book Company, New York & London.
Kerrigan, R. W. (1995): *Global genetic resources for Agaricus breeding and cultivation*, Can. J. Bot. 73 (Suppl. 1): S973—S979.
Nanagulyan, S. G., Yesayan, A. G. (2002): *Fungal diseases of cultivated mushrooms in Armenia*, Book of Abstracts, 7th International Mycological Congress. Oslo, Norway, pp. 259.
Potočnik, I. (2006): *Prouzrokovači truleži šampinjona (Agaricus bisporus (Lange) Imbach) i njihova osetljivost na fungicide*, magistarska teza, Biološki fakultet, Univerzitet u Beogradu.
Sharma, S. R., Kumar, S. (2000): *Studies on wet bubble disease of white button mushrooms (Agaricus bisporus) caused by Mycogone perniciosa*, U: *Mushroom Science XV. Science and Cultivation of Edible Fungi*. Ed. Van Griensven, L. J. L. D., Balkema, Rotterdam, pp. 569—575.
Sisto, D., Faggiano, S., Rana, G. L. (1997): *Mycogone perniciosa, a potential threat for cultivated mushrooms in southern Italy*, Petria 7 (3): 159—164.
Smith, D. E. V. (1924): *Three diseases if cultivated mushrooms*, Transactions of the British Mycological Society 10: 81—97.
Umar, M. H., Geels, F. P., Van Griensven, L. J. L. D. (2000): *Pathology and pathogenesis of Mycogone perniciosa infection of Agaricus bisporus*. U: *Mushroom Science XV. Science and Cultivation of Edible Fungi*, Ed. Van Griensven, L. J. L. D., Balkema, Rotterdam, pp. 561—567.
Webster, J. (1970): *Coprophilous fungi*, Trans. Br. Mycol. Soc. 54: 161—180.

МОРФО-ФИЗИОЛОШКЕ КАРАКТЕРИСТИКЕ И ИНТЕРАКЦИЈЕ ИЗОЛАТА *MYCOGONE PERNICIOSA* (MAGNUS) DELACR.

Јасмина М. Гламочлија[1], Марина Д. Соковић[1],
Милица В. Љаљевић-Грбић[2], Јелена Б. Вукојевић[2],
Иванка М. Миленковић[2], Л. Ј. Л. Д. ван Гриенсвен[3]
[1] Институт за биолошка истраживања „Синиша Станковић",
Булевар деспота Стефана 142, Београд, Србија
[2] Институт за ботанику, Биолошки факултет,
Универзитет у Београду Таковска 43, Београд, Србија
[3] Plant Research International, Wagenningen University, The Netherlands

Резиме

Mycogone perniciosa (Magnus) Delacr., изазивач обољења мокре трулежи, најчешћи је узрочник губитака у гајилиштима *Agaricus bisporus* Lange (Imb) у Србији. Извршена је изолација и идентификација 5 изолата *M. perniciosa* са оболелих плодоносних тела шампињона из гајилишта у Србији, Босни и Херцеговини и Холандији. Испитиване су морфо-физиолошке карактеристике као и степен сродности проучаваних изолата на основу анализе међусобног деловања колонија, односно коришћењем феномена хифалне интерференције. Макроскопска и микроскопска истраживања односа изолата и добијени резултати указују да хифална интерференција може представљати додатни параметар у разликовању изолата *M. perniciosa*. Изолати добијени из гајилишта у Србији слични су међусобно, као и изолати из Холандије који су показали међусобну сличност али се разликују од претходних. Изолати из Босне и Херцеговине разликовали су се и од српских и од холандских.