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GIBBERELLA INTERMEDIA THE PATHOGEN OF ST. JOHN'S WORT, CONEFLOWER AND MARSHMALLOW IN SERBIA

ABSTRACT: *Gibberella intermedia* (Kuhlmann) Samuels et al. (anamorf: *Fusarium proliferatum* /Matsushima/ Nirenberg) was isolated from seeds of St. John's wort, marshmallow, and coneflower, as well as from roots and stalks of marshmallow and roots of coneflower. These plants had symptoms of leaf chlorosis, malformation, withering and plant dwarfing and were collected from several localities in Serbia during five-year investigations of mycopopulations of the mentioned plants.

The morphological characteristics of the pathogen were described.

KEY WORDS: medicinal plants, St. John's wort, marshmallow, coneflower, *Gibberella intermedia*

INTRODUCTION

Medicinal plants are among economically most significant plants in Serbia. There are over 700 species of these plants on the territory of Serbia, contributing 19,65% of the total flora (Sarić, 1989). Out of 420 registered species, 279 are used commercially as medicinal and aromatic plants. In order to preserve medicinal plants in nature from overexploitation, they are grown as commercial crops in plantation. There is a long tradition of growing medicinal and aromatic herbs commercially such as mint, marshmallow, sage, St. John's wort, coneflower, etc. in Serbia. The commercial cultivation led to the occurrence and spread of the plant pathogens with epidemic distribution on some hosts (St. John's wort, marshmallow, and coneflower).

During the experimental research of mycopopulation of medicinal herbs, there was a total of 7 *Fusarium* species identified: 6 species on St. John's wort, 5 species on *Althea officinalis*, 3 on *E. angustifolia* and 5 on *E. purpu-*

rea (Pavlović et al., 2008). *F. proliferatum* /Matsushima/ Nirenberg was identified on all three hosts. This research was conducted to investigate basic morphological, cultural and pathogenic characteristics of *Gibberella intermedia* (Kuhlmann) Samuels et al. (anamorph: *Fusarium proliferatum* /Matsushima/ Nirenberg) isolates.

MATERIALS AND METHODS

Collection and preparation of the samples: The samples of diseased plants of St. John's wort, marshmallow and coneflowers were collected from the medicinal plant collection of the Institute in Pančevo vicinity, as well as from the cultivation fields of cooperatives in several localities of Serbia (Zrenjanin, Ruma, Inđija, Pančevo, Kačarevo) over the period 2002—2006. The plants showing the symptoms of withering, dwarfness, chlorosis and leaf shrivelling were collected monthly from March to November. Samples were washed with tap water, disinfected with 2% NaOCl for 30 seconds and washed again with sterile water.

The methods that were used: The standard procedure of Dhingra and Sinclair (1986) was used for isolation of fungi from the tissue of leaf petioles, stems, roots and seeds of diseased plants on carrot agar (CA) and carnation leaf agar (CLA). A modified method of Leslie (1991) was used to investigate teleomorph development. The fragments of diseased tissue were incubated on CA at 25±2°C. After 2—4 days, the existing mycelia were transferred on the 60 mm Petri dishes with potato dextrose agar (PDA) to obtain a pure culture.

The morphological characteristics of isolates were studied on potato dextrose agar (PDA), synthetic nutrition agar (SNA), carrot agar (CA) and carnation leaf agar (CLA), prepared according to directions supplied by Booth (1971), Muntanola-Cvetković (1982), Fisher et al. (1982), Leslie (1991), Leslie and Summerell (2006) and Lević, (2008).

The physiological characteristics (the growth of selected isolates) were tested on PDA at 25 and 30°C. The colony diameter was measured after 73 hours of incubation, as suggested by Burgess et al. (1994) and the results represented the mean of three replicates.

The identification of the pathogen was based on morphological and physiological characteristics of the isolates (appearance of aerial and substrate mycelia, morphology of macro- and micro conidia, the colony growth on PDA at 20°C, and pigmentation of the medium). The taxonomic keys of Booth (1971), Gerlach and Nirenberg (1982), Nelson et al. (1983), Burgess et al. (1994), Summerell et al. (2002), Leslie and Summerell (2006), Dugan (2006) and Lević (2008), were used for the identification of the obtained isolates.

The pathogenicity was confirmed by the modified method of Molt and Simone (1967). Four hundred seeds of each medicinal plant were sown in plastic pots with sterile sand. The pots were irrigated with 100 ml of conidial suspension, prepared from 7-day old culture grown on PDA. After 21 days the

seedlings were picked and washed in distilled water. The level of root necrosis was calculated according to the scale from 0 to 3 (0 — health seedling; 1 — root tip necroted; 2 — root and down part of stem necroted; 3 — whole seedling necroted).

RESULTS

Collection of isolates

Gibberella intermedia was isolated from seeds of all tested medicinal plants, as well as roots and stems of marshmallow, and roots of *Echinacea purpurea*. The pathogen caused symptoms of seedlings decay and root rot, which resulted in suppressed growth, chloroses and malformation of diseased plants. From around 200 isolates, 29 of them were selected for further investigations: 15 were obtained from seeds, 8 from roots, 3 from stalks and 3 from seedlings. From marshmallow, coneflower (*Echinacea purpurea* and *E. angustifolia*) and St. John's wort 10, 15 (10 + 5), and 4 isolates were obtained, respectively (Table 1).

Tab. 1 — The list of *Gibberella intermedia* isolates used in the study

Host	Isolates	Place of isolation	Locality
Althaea officinalis	S-4	stalk	Zrenjanin
	S-7	seed	Pančevo
	S-8	seed	Pančevo
	S-10	root	Zrenjanin
	S-13	root	Zrenjanin
	S-18	root	Zrenjanin
	S-20	seed	Pančevo
	S-30	root	Ruma
	S-35	root	Zrenjanin
	S-38	stalk	Zrenjanin
Echinacea purpurea	E-67	seedlings	Ruma
	E-44	seedlings	Ruma
	E-7	seed	Pančevo
	E-202	root	Inđija
	E-204	root	Inđija
	E-253	seed	Pančevo
	E-263	stalk	Ruma
	E-266	seed	Pančevo
	E-400	seed	Pančevo
	E-404	root	Inđija
Echinacea angustifolia	Ea-31	seedlings	Ruma
	Ea-34	seed	Pančevo
	Ea-77	seed	Pančevo
	Ea-78	seed	Pančevo
	Ea-19	seed	Pančevo
Hypericum perforatum	K-77	seed	Pančevo
	K-79	seed	Kačarevo
	K-80	seed	Kačarevo
	K-82	seed	Kačarevo

Morphological characteristics

Mycelium was abundant, woolly, whitish, and with age it became light to dark violet. Colour of mycelium and medium pigmentation varied upon the isolate (Fig. 1).

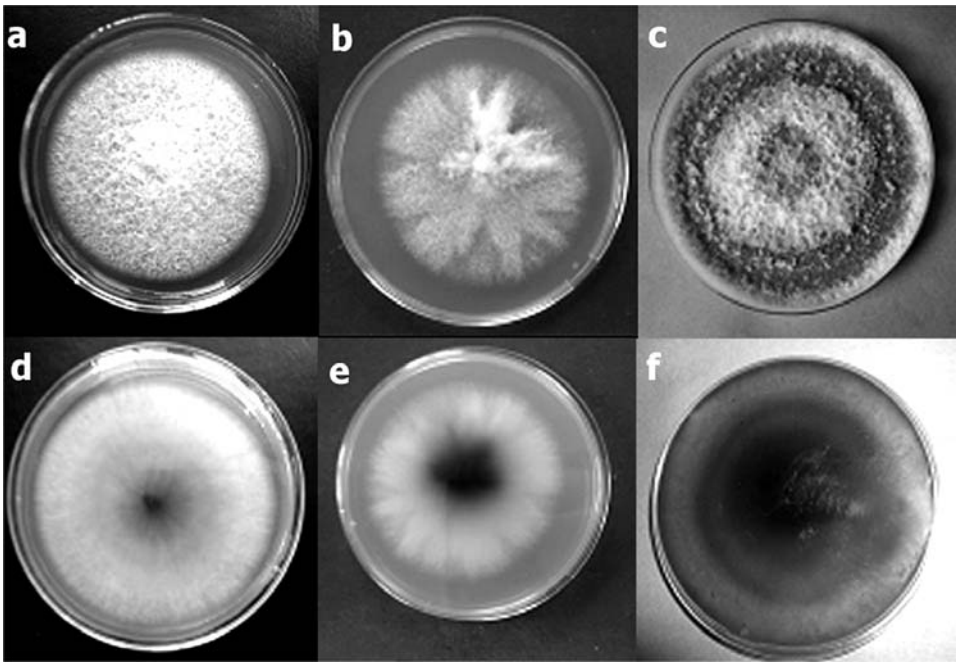

Fig. 1 — Appearance of aerial (upper row) and substrate mycelia (lower row) of *Gibberella intermedia* isolates E-404 (a, d), K-79 (b, e) and S-18 (c, f)

On infected germinating seeds, incubated on wet filter paper, mycelium was powdery, white, almost entirely covering the infected seeds. Such seeds did not germinate and they completely decayed. The filter paper under these seeds was coloured ink-blue. *Ascocarp* was perithecia, spherical or obovoid, black, 210—390 mm in diameter on CA. *Ascuses* were elongated, hyaline, with 4—8 ascospores, 66,5—88,0 x 8,50—14,20 mm. *Ascospores* were with 2—3 septa, straight or slightly narrowed by septa, hyaline, 15—17,5 x 5,0 mm. *Microconidia* were formed in chains or "false heads" on polyphialides and monophyalides. They were oval with rounded base, or rarely two-celled, hyaline, 3,0—19,5 x 2,0—5,0 mm. *Macroconidia* were formed on monophyalides on branched conidiophores in sporodochia, rarely on monophyalides on hyphae. The abundant pale orange sporodochia formed on CLA under combination of fluorescent and UV light. They were slender, falcate or straight, thin-walled, mostly with 3—5 septa, the basal cell pediculate, 16,5—73,0 x 2,5—5,0 mm (Figure 2). There were some variation in conidia dimensions depending on isolate and host. *Hlamydospores* were absent.

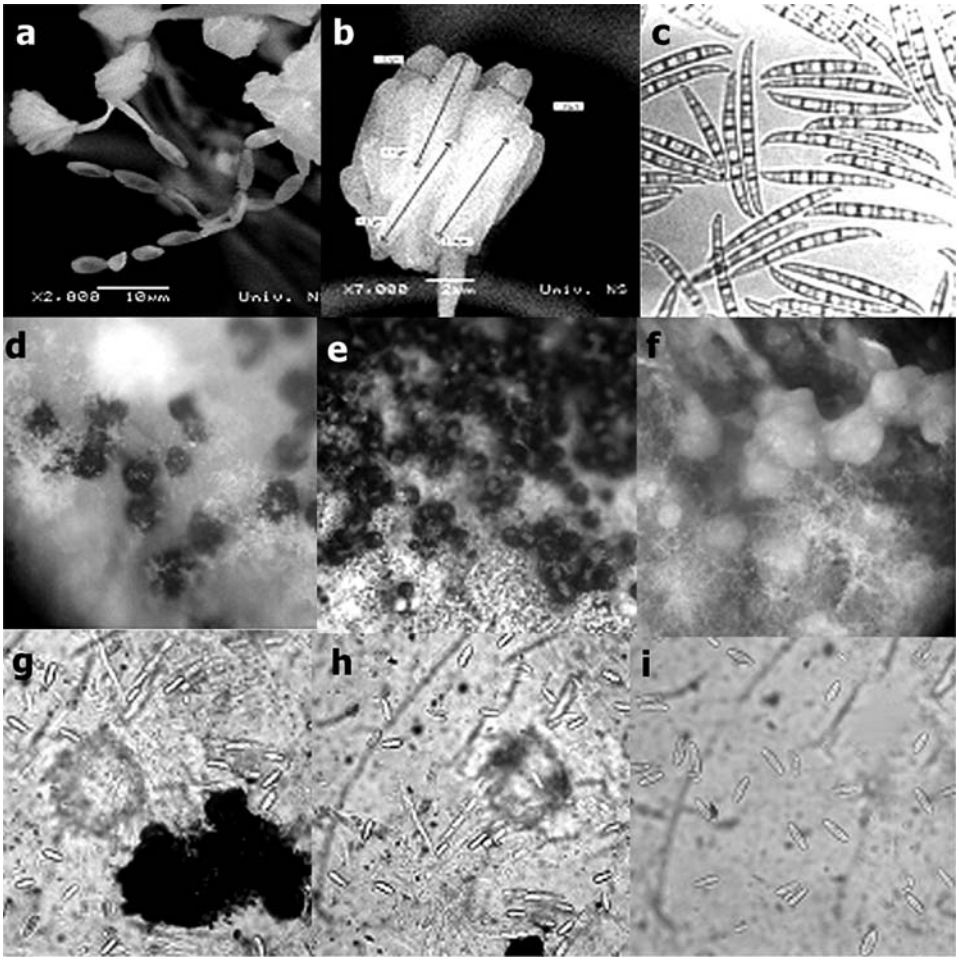

Fig. 2 — *Gibberella intermedia*: microconidia in long chains (a) and "false heads" (b) (SEM); macroconidia from sporodochia (c); perithecia of *Gibberella intermedia* obtained by crossing isolates (d, e); leaking fertile perithecia (f), ascuses (g, h) and ascospores (i)

Physiological characteristics

The uniform growth of isolates at 25 and 30°C was observed. However, the growth of the isolates from marshmallow was faster at 30°C (Table 2). There were no statistically significant differences in average values of growth of all isolates from the investigated medicinal plants at 25 and 30°C (34,1 mm and 34,5 mm, respectively) ($LSD_{0,05}$ = 3,24, $LSD_{0,01}$ = 5,95).

Tab. 2 — Radial growth of colonies of *G. intermedia* isolates from marshmallow, St. John's wort and coneflower on PDA at 25 and 30°C after three days (in mm)

Host	Isolates	Colony growth at	
		25°C	30°C
Althaea officinalis	S-4	32.7 d[y]	33.0 d
	S-7	35.7 bc	36.0 c
	S-8	31.0 e	35.7 c
	S-10	35.7 bc	37.7 b
	S-13	36.7 b	39.7 a
	S-18	35.0 c	35.7 c
	S-20	38.7 a	40.7 a
	S-30	34.7 c	37.0 bc
	S-35	32.0 de	37.0 bc
	S-38	37.0 b	39.7 a
	Average[z]	34.9 B	37.2 A
Hypericum perforatum	K-77	36.0 a	33.0 ab
	K-79	32.0 a	32.0 b
	K-80	36.7 a	34.0 a
	K-82	28.7 b	25.7 c
	Average	33.4 A	31.2 A
Echinacea purpurea	E-67	35.0 a	34.0 bcd
	E-44	32.0 c	34.7 ab
	E-7	34.0 ab	36.0 a
	E-202	35.0 a	36.0 a
	E-204	33.7 ab	32.3 ce
	E-253	35.0 a	35.3 ab
	E-263	32.7 bc	34.0 bcd
	E-266	34.7 a	34.0 bc
	E-400	35.0 a	36.3 a
	E-404	35.0 a	36.3 a
	Average	34.2 A	34.9 A
Echinacea angustifolia	Ea-31	34.0 a	34.3 abc
	Ea-34	34.0 a	34.0 bc
	Ea-77	33.0 a	33.3 c
	Ea-78	33.0 a	35.3 ab
	Ea-19	34.0 a	36.0 a
	Average	33.6 A	34.6 A

[y] Values in the columns designated by the same letter are not statistically significantly different on the basis of Duncan's test (P = 0,05).

[z] Average values of growth of all isolates under tested temperatures for the same host, designated by the same capital letter are not statistically significantly different on the basis of Duncan's test.

Pathogenicity of tested isolates

All isolates of *G. intermedia* caused root necrosis of seedlings. There were no significant differences between the isolates, but the isolates Ea-77 and Ea-78 showed lower pathogenicity in comparison with other isolates (Table 3).

Tab. 3 — Effect of different *G. intermedia* isolates on seedling root necrosis of tested medicinal plant species under laboratory conditions

Host	Isolates	Root necrosisz
Althaea officinalis	S-7	3,0 a
	S-10	2,0 a
	S-18	3,0 a
	S-30	2,0 a
	S-35	3,0 a
	check	0,0 b
Echinacea purpurea	E-7	2,0 a
	E-44	3,0 a
	E-204	2,0 a
	E-266	3,0 a
	E-400	2,0 a
	check	0,0 b
Echinacea angustifolia	Ea-19	2,7 a
	Ea-31	3,0 a
	Ea-34	3,0 a
	Ea-77	2,0 b
	Ea-78	1,0 c
	check	0,0 b
Hypericum perforatum	K-77	3,0 a
	K-79	2,0 a
	K-80	3,0 a
	K-82	2,0 a
	check	0,0 b

z Values in the columns designated by the same letter are not statistically significantly different on the basis of Duncan's test (P = 0,05).

DISCUSSION

Morphological, cultural and pathogenic characteristics of the tested isolates were described according to data for *G. intermedia* given in the literature (Booth, 1971; Gerlach and Nirenberg, 1982; Nelson et al., 1983; Burgess et al., 1994; Leslie and Summerell, 2006). This species is known as a pathogen found on seeds of a number of cultivated plant species, such as wheat, corn, broomcorn, sugar beet, sunflower and soybean (Jovičević and Milošević, 1990; Summerell et al., 2002; Lević et al., 2003; Leslie and Summerell, 2006; Lević, 2008), and it causes a decrease of germination and germination energy, wilting and seedling decay, known as firing and melting of seedlings (Jasnić and Maširević, 2006). Anamorph of this species (*F. proliferatum*) was already identified on seeds and seedlings of coneflower (Pavlović et al., 2006, 2006a), and seeds, roots, and lower part of stalks of marshmallow (Pavlović and Stojanović, 2002, Pavlović et al., 2007).

The results of the conducted study, which was preferentially diagnostic in nature, will allow more comprehensive recognition of mycoflora of medicinal and aromatic plants in our conditions. These results will also enable more ef-

fective protection of medicinal and aromatic plants, i.e. improved and more profitable cultivation.

REFERENCES

Booth, C. (1971): *The Genus Fusarium*, Commonwealth Mycological Institute, Kew, Surrey, England, 1—237.

Burgess, L. W., Summerell, B. A., Bullock, S., Gott, K., Backhouse, D. (1994): *Laboratory Manual for Fusarium Research*, Ed 3, *Fusarium* Research Laboratory, Department of Crop Science, University of Sydney and Royal Botanic Gardens, Sydney, 1—133.

Dingra, O. D., Sinclair, J. B. (1986): *Basic Plant Pathology Methods*, CRC Press Inc., Boca Raton, Florida, USA, 1—355.

Dugan, F. (2006): *The identification of fungi*, APS, Minnesota, 176.

Fisher, N. L., Burgess, L. W., Tiussoun, T. A., Nelson, P. E. (1982): *Carnation leaves as a substrate and for preserving cultures of Fusarium species*, Phytopathology, 72, 151—153.

Gerlach, W., Nirenberg, H. (1982): *The Genus Fusarium and Pictorial Atlas, Biologische Bundes-Anstalt für Land und Forstwitschaft*, Institut für Microbiologie, Berlin-Dahlen, 209.

Jasnić, S., Maširević, S. (2006): *Bolesti klijanaca suncokreta*, Biljni lekar, 34: 313—315.

Jovičević, B., Milošević, M. (1990): *Bolesti semena*, Dnevnik, Novi Sad, 1—292.

Leslie, J. (1991): *Mating populations in Gibberella fujikuroi (Fusarium Section Liseola)*, Phytopathology, 81, 1059—1060.

Leslie, J., Summerell, B. (2006): *The Fusarium Laboratory Manual*, Blackwell Publishing, 388.

Lević, J. (2008): *Vrste roda Fusarium*, Institut za kukuruz, Beograd, Društvo genetičara Srbije, 1226.

Lević, J., Ivanović, D., Stanković, S. (2003): *Paraziti kukuruza, sirka i prosa koji se prenose semenom*, Biljni lekar, 31, 570—575.

Molt, P. M., Simone, J. (1967): *Technique de dentamination artificiele des semences de Mais par les Fusarioses*, Revue de Zoologie Agricole at Appliquie, No. 1—3, 29—32.

Muntanola-Cvetković, M. (1982): *Opšta mikologija*, NIRO Književne novine, Beograd, 13—320.

Nelson, P. E., Toussoun, T. A., Marasas, W. F. O. (1983): *Fusarium species. An illustrated manual for identification*, The Pennsylvania State University Press, University Park and London, 1—193.

Pavlović, S., Stojanović, S. (2002): *Mycoflora of marshmallow (Althaea officinalis L.)*, The 2nd Conference on Medicinal and Aromatic Plants of Southeast European Countries, Chalkidiki, Greece, Book of abstracts, 134.

Pavlović, S., Stojanović, S., Starović, M. (2006): *Current study on parazitic microorganisms of medicinal and aromatic plants in Serbia*, Proceedings of the 4th Conference on Medicinal and Aromatic Plants of Southeast European Countries, Iasi, Romania, 214—219.

Pavlović, S., Stojanović, S., Starović, M. (2006a): *Diseases of purple coneflower seeds*, Procedings from 3rd Conference on Medicinal and Aromatic Plants of Southeast Erupoean Countries, Nitra, Slovac Republic, 140—144.
Pavlović, S., Stojšin, V., Stojanović, S. (2007): *Mycopopulation of marshmallow (Althaea officinalis L.)*, Zbornik Matice Srpske, 113, 193—202.
Pavlović, S., Stojšin, V., Stojanović, S., Starović, M. (2008): *Mycopopulation in five important cultivated medicinal plants in Serbia*, Proceedings of the 5th CAMAPSEEC, Brno, Czech Republic, 04—14:1—7.
Sarić, M. (1989*): Lekovite biljke Srbije*, SANU, Beograd, 640.
Summerell, B., Nirenberg, H., Seifert, K. (2002): *Perithecial species of Fusarium*, Usummerell, B., Leslie, J., Backhouse, D., Bryden, W., Burgess. L., Fusarium, Ed 2, APS, Minnesota, 1—14.

GIBBERELLA INTERMEDIA ПАТОГЕН КАНТАРИОНА, ЕХИНАЦЕА И БЕЛОГ СЛЕЗА У СРБИЈИ

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Резиме

Gibberella intermedia (Kuhlmann) Samuels et al. (анаморф: *Fusarium proliferatum* /Matsushima/ Nirenberg) је констатована на белом слезу, ехинацеи и кантариону и то на семену, корену и стаблу, као и на расаду ехинацеа. Основни симптом заражених биљака *G. intermedia* су били: пропадање клијанаца, трулеж корена и кореновог врата, патуљавост, асиметричан пораст, увелост, хлороза и смежураност листова. Узорци зараженог биљног материјала били су пореклом из околине Панчева, Зрењанина, Банатског Новог Села, Инђије, Руме и Старе Пазове. У раду су дате морфолошке, физиолошке и патогене карактеристике изолата *G. intermedia*.