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PARASITE FUNGI ON SOME ORNAMENTAL IRIDACEAE (II)

Oana ROŞCA-CASIAN, Marcel PÂRVU
Universitatea "Babeş-Bolyai", Facultatea de Biologie şi Geologie, Catedra de Taxonomie şi Ecologie,
str. Republicii, nr. 42, **RO-400 015 Cluj-Napoca**
e-mail: casioana@yahoo.com

Abstract: *Botryotinia draytonii, Fusarium oxysporum* f. sp. *gladioli* and *Heterosporium pruneti* fungi were isolated from ornamental Iridaceae and were obtained in pure cultures on nutritive medium. The fungal sporulation was examined by means of the light microscope and/or scanning electron microscope.

Key words: *Botryotinia draytonii,* fungal colony, *Fusarium oxysporum* f. sp. *gladioli, Heterosporium pruneti,* Iridaceae, micrograph, microfungi, mycelium, *Penicillium gladioli,* sporulation

Introduction

Literature data mention numerous main and/or occasional parasite fungi on ornamental Iridaceae. The main parasite fungi on *Freesia hybrida* are *Cladosporium herbarum* and *Fusarium oxysporum* f. sp. *gladioli*, on *Gladiolus hybridus* are *Septoria gladioli, Botryotinia draytonii* (syn. *Botrytis gladiolorum*) and *Fusarium oxysporum* f. sp. *gladioli*, and on *Iris germanica* are *Puccinia iridis, Colletotrichum dematium* (syn. *Vermicularia dematium*), *Heterosporium pruneti* and *Botrytis cinerea* [4, 9, 11, 15].

Stromatinia gladioli (syn. *Sclerotinia gladioli*), *Septoria gladioli, Cladosporium iridis, Gibberella moniliformis* (syn. *Fusarium moniliforme*) [4] and *Botrytis cinerea* [4, 15] are mentioned as occasional parasite fungi on *Freesia hybrida*, *Urocystis gladiolicola* (syn. *Urocystis gladioli*) and *Mystrosporium polytrichum* on *Gladiolus hybridus* [15], *Cladosporium herbarum, Cladosporium iridis* and *Ramularia rollandi* on *Iris germanica* [4], and *Puccinia croci* on *Crocus* [4].

This study was conducted by the fact that ornamental value of Iridaceae is mostly affected by mycoses. Besides the before identified fungi (*Fusarium oxysporum* f. sp. *gladioli*, *Penicillium gladioli, Botrytis cinerea*) [12], there were also isolated *Botryotinia draytonii* and *Heterosporium pruneti*.

Materials and Methods

Botryotinia draytonii was isolated from *Gladiolus hybridus* flowers and *Heterosporium pruneti* from *Iris spuria* ssp. *ochroleuca* leaves. These fungi were cultivated, by central point inoculation, on Czapek-agar nutritive medium, prepared according to literature [5, 10]. The pure fungal colonies were obtained by repeated isolations, and they were incubated at 22 ºC and were observed at equal periods of time (3, 6, 9, and 12 days), regarding the mycelial growth and the formation of sporulation and/or sclerotia on nutritive medium from Petri plates (70 mm in diameter). Six repetitions were done for each isolated fungus. The data were analyzed and standard error was calculated. The colony's diameter and the formation of sporulation and/or sclerotia were estimated according to species.

The identification of isolated fungi in "Al. Borza" Botanical Garden from Cluj-Napoca was done by examination of sporulation by means of light microscope and/or JEOL JSM 5510 LV scanning electron microscope. Preparation and examination of fungi (*Botryotinia draytonii* from *Gladiolus hybridus* and *Fusarium oxysporum* f. sp. *gladioli* from *Freesia hybrida*) were

done according to the methodology from the literature [7], at the Center for Electron Microscopy of "Babeş-Bolyai" University Cluj-Napoca.

Results and Discussions

A very common disease on *Gladiolus hybridus* plants in Al. Borza" Botanical Garden from Cluj-Napoca is induced by *Botryotinia draytonii* fungus, which causes gray mould. The attack is frequently encountered on flowers, but is also present on leaves, shoots and bulbs [15].

Botryotinia draytonii formed mycelium, gray sporulation and sclerotia on Czapek-agar nutritive medium. The fungal colony had about 28 mm, 3 days after inoculation, 65 mm after 6 days, and it occupied the whole Petri plate, 9 days after inoculation (**Table 1**). The colony had gray sporulation and black mature sclerotia, 9 days after inoculation.

Botryotinia draytonii sporulation is made up of brown, septated, branched conidiophores, with sterigmae on which ovoid, yellowish conidia (8.7–12.5 × 5–7.5 µm) are formed [15].

In micrographs obtained by scanning electron microscope, *Botryotinia draytonii* conidia have ovoid shape and present numerous short protuberances on their surface (**Fig. 1**). The obtained results are according to literature data, which state that the surface of dry *Botrytis* conidia has numerous short protuberances, which disappear after hydration and redrying [6].

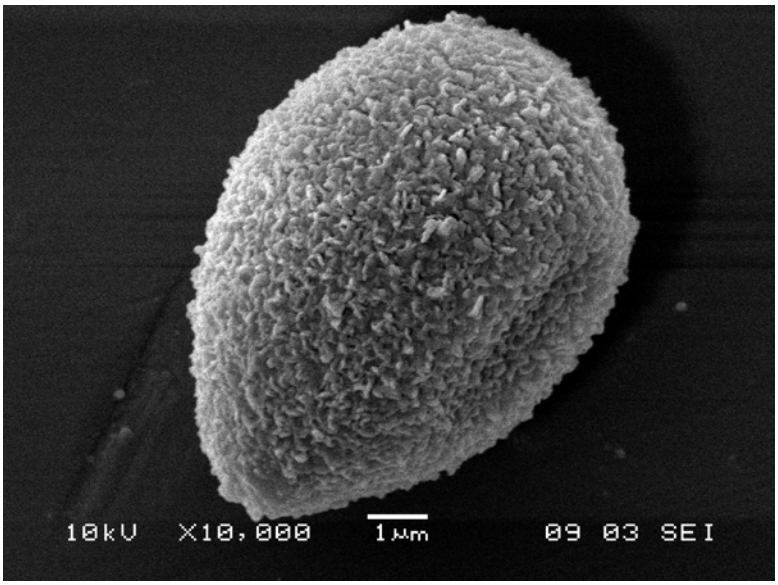


Fig. 1: Scanning electron micrograph of *Botryotinia draytonii* conidium with short protuberances

Literature data mention that *Fusarium oxysporum* f. sp. *gladioli* has microconidia variable in size and shape, from ovoid to cylindrical, straight or slightly curved, of 5-12 × 2.2-3.5 µm. Macroconidia are 3(5)-septate, fusiform, more or less curved, of 27-46 × 3-4.5 µm. Chlamidospores are in hyphae or conidia, are globosely, have 5-15 µm in diameter, are smooth or rough walled, formed intercalary or at the end of hyphae and are solitaire, paired or in chains [14].

Scanning electron micrographs of *Fusarium oxysporum* f. sp. *gladioli* sporulation show mycelial hyphae, unicellular, ovoid microconidia and fusiform, 3-4 septate macroconidia (Fig. 2). Conidia's surface is smooth and the septa are obvious (Fig. 3). Chlamidospores are spherical, rough-walled, formed intercalary on hyphae, have approximately 8-10 µm in diameter, and are solitaire (Fig. 4).

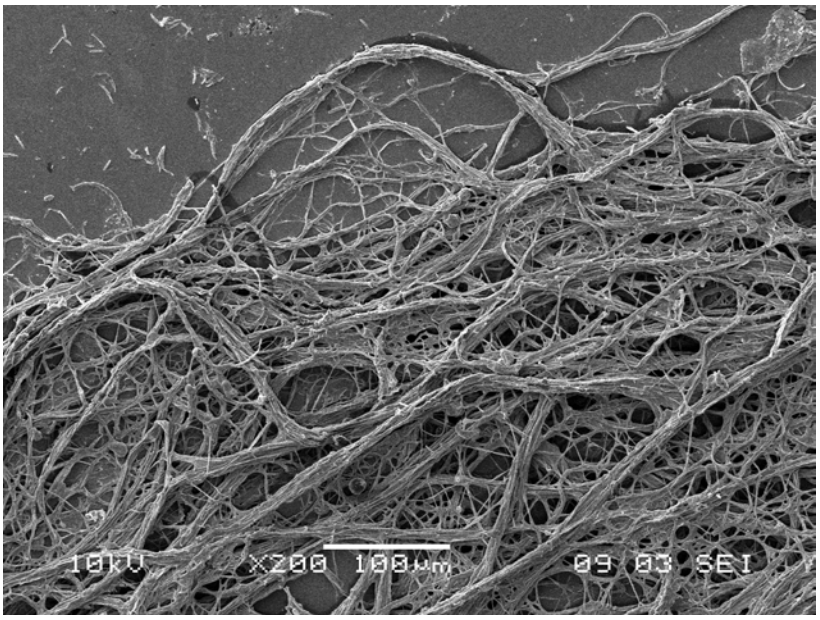


Fig. 2: Scanning electron micrograph of *Fusarium oxysporum* f. sp. *gladioli* mycelium and sporulation (microconidia and macroconidia)

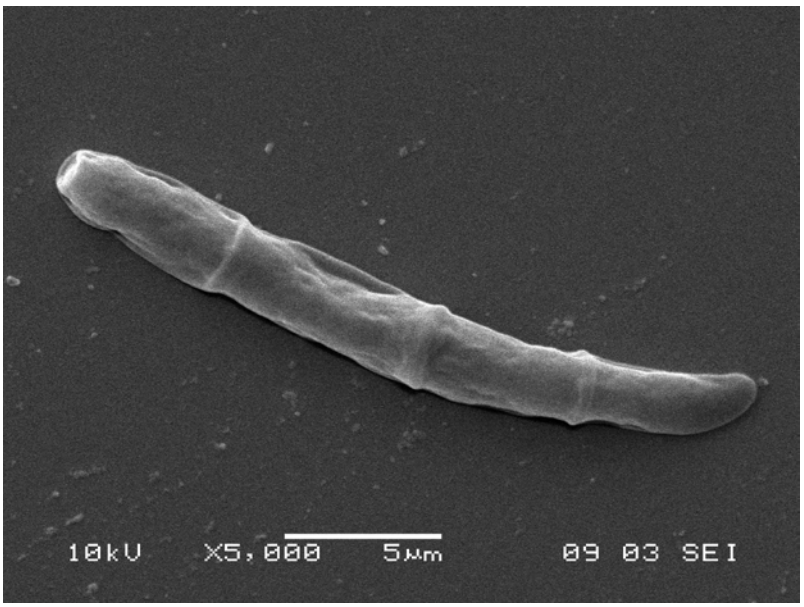


Fig. 3: Scanning electron micrograph of *Fusarium oxysporum* f. sp. *gladioli* septate macroconidium

Heterosporium pruneti fungus has a slower growth on Czapek-agar nutritive medium, compared to *Botryotinia draytonii*. Thus, the colony has brown mycelium and 6 mm in diameter, 3 days after inoculation, 15 mm after 6 days, and about 30 mm after 12 days (Table 1).

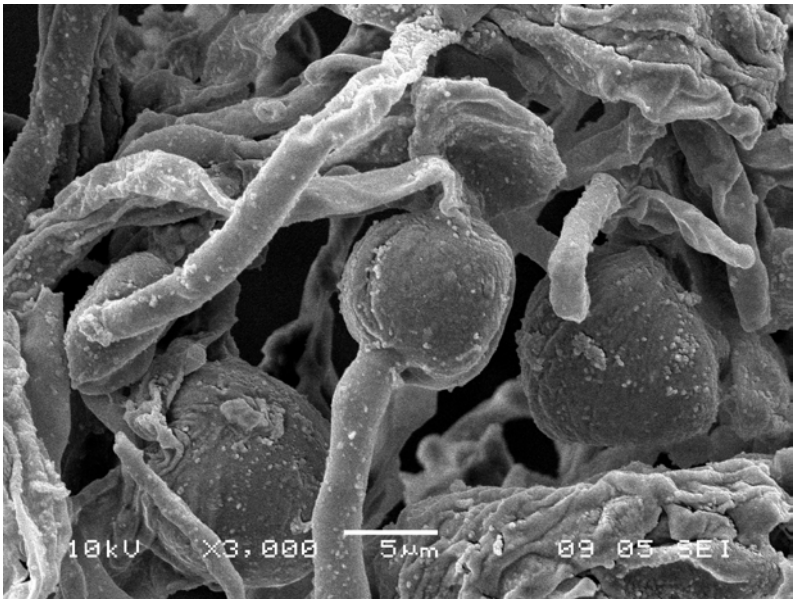


Fig. 4: Scanning electron micrograph of *Fusarium oxysporum* f. sp. *gladioli* chlamidospores

Table 1: The effect of Czapek-agar nutritive medium on "in vitro" growth of *Botryotinia draytonii* and *Heterosporium pruneti* fungi, isolated from ornamental Iridaceae

Fungus name	Colony's diameter (mm) and Standard error (SE) after							
	3 days	SE	6 days	SE	9 days	SE	12 days	SE
Botryotinia draytonii	28	±0.718	67	±0.600	70	±0.333	70	0
Heterosporium pruneti	6	±0.421	15	±0.683	23	±0.421	30	±0.494

Heterosporium pruneti is a frequently encountered fungus on *Iris* plants in "Al. Borza" Botanical Garden from Cluj-Napoca, and causes leaves spotting and drying. The disease begins by the appearance of ovoid spots, of different size, often disposed on the edge of the leaves. At the beginning, the spots are yellowish, then turn brown and are limited by a reddish zone. Fungus brown sporulation is formed on spots and is made up of septate, unbranched conidiophores, grouped in bunches and cylindrical, septate, echinulated conidia (38 – 55 × 14-18 µm) [15].

Different methods are used for the protection of ornamental plants against *Botryotinia draytonii* and *Heterosporium pruneti* fungi.

Gladiolus gray mould is transmitted from one plant to another by attacked bulbs and plant debris. The disease attack is more intense in wet years. Different biological substances (Trichodermin, Trichodermicin, Trichosemin etc.) obtained from *Trichoderma viride* (sporulation made up of branched conidiophores with long, main branches, and short and fewer secondary branches) are used for the biological control of this disease [2, 3].

The enzymes have an important role in the interaction between *Botrytis* species and *Trichoderma viride* hyperparasite fungus, but there are also physical interactions like the direct penetration of pathogenic cells by *Trichoderma* fungus [13].

Meaningful results were achieved regarding the chemical control of gray mould caused by *Botrytis* species, with different fungicides such as Ronilan 50 WP (vinclozolin 50 %) 0.10 %; Ridomil 25 WP (mefenoxam 4 % + mancozeb 64 %) 0.25 %; Captan 50 WP (captan 50 %) 0.20 %; Topsin 70 PU (tiophanate methyl 70 %) 0.10 %; Bravo 500 SC (chlorothalonil 500 g/l) 0.20 %; Captadin 50 PU (captan 50 %) 0.20 %, etc. [1, 10].

The chemical control of *Heterosporium pruneti* fungus was done by using fungicides like Mancozeb 80 (mancozeb 80 %) 0.2 %; Zineb 75 (zineb 75 %) 0.20 %; Bordeaux mixture (metallic copper 20 %) 0.50 %, etc. [8, 15].

Conclusions

- The main mycoses identified on ornamental Iridaceae (*Freesia, Gladiolus, Crocus,* and *Iris*)in "Al. Borza" Botanical Garden from Cluj-Napoca are caused by *Fusarium oxysporum* f. sp. *gladioli*, *Penicillium gladioli*, *Botrytis cinerea, Botryotinia draytonii*, and *Heterosporium pruneti*;
- *Botryotinia draytonii* conidia examined by means of scanning electron microscope present short protuberances on their surface;
- Scanning electron micrographs of *Fusarium oxysporum* f. sp. *gladioli* showed mycelian hyphae, ovoid microconidia, fusiform, septate macroconidia and solitaire, rough-walled chlamidospores.

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CIUPERCI PARAZITE PE UNELE IRIDACEAE ORNAMENTALE (II)

(Rezumat)

În acest studiu s-a pornit de la ideea că valoarea ornamentală a iridaceelor este afectată cel mai frecvent de micoze. *Fusarium oxysporum* f. sp. *gladioli*, *Penicillium gladioli*, *Botrytis cinerea*, *Botryotinia draytonii* şi

Heterosporium pruneti sunt cele mai frecvente ciuperci întâlnite pe plantele de *Gladiolus hybridus*, *Freesia hybrida* şi *Iris germanica* din Grădina Botanică "Al. Borza" din Cluj-Napoca.

Pe lângă ciupercile izolate şi identificate anterior (*Fusarium oxysporum* f. sp. *gladioli*, *Botrytis cinerea*, *Penicillium gladioli*), au fost izolate şi speciile *Botryotinia draytonii* şi *Heterosporium pruneti*. Acestea au fost obținute în culturi pure pe mediul nutritiv Czapek-agar, iar pentru identificare, sporulația fungică a fost examintă la microscopul optic şi/sau microscopul electronic scanning.