DIVERSITY OF FUNGI ASSOCIATED WITH *RIBES NIGRUM* L. CROP IN THE SOUTH OF ROMANIA

DIVERSITATEA CIUPERCILOR ASOCIATE CULTURII DE *RIBES NIGRUM* L. ÎN SUDUL ROMÂNIEI

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Abstract: *Black currant (Ribes nigrum L.) there is one of the most valuable medicinal plants for the nourishment and therapeutically value of fruits, buds, leaves and seeds. From the organs of the black currant there have been isolated some species of pathogenic fungi and some mycotoxicogenic fungi which could alter the quality of the raw material. Fungi with an antagonistic potential were identified too. This isolates of fungi may be further investigated in order to establish which of them will be suitable for biological control of the black currant culture.*
Key words: medicinal plants, phytopathogenic fungi, antagonistic fungi, phylloplane

Rezumat: *Coacăzul negru (Ribes nigrum L.) este unul dintre arbuştii fructiferi extrem de apreciați pentru valoarea alimentară şi terapeutică a fructelor, mugurilor, frunzelor şi semințelor. De pe organe ale plantei au fost izolate specii de ciuperci saprofite potențial patogene, dar şi unele ciuperci micotoxicogene care ar putea deprecia calitatea materiei prime. Au fost identificate şi specii de ciuperci cu potențial antagonist. Aceste izolate de ciuperci ar putea fi investigate ulterior pentru a stabili care dintre acestea să fie folosite în controlul biologic al culturii de coacăz negru.*
Cuvinte cheie: plante medicinale, ciuperci patogene, ciuperci antagoniste, filoplan

INTRODUCTION

Black currant (*Ribes nigrum* L.) is a highly valuable medicinal plant. Fruits and buds, leaves, seeds, are used for their therapeutic value (Bojor, 2003, Raiciu, 2011).

Currant plantation is affected by a large number of pathogens, especially fungi (Rădulescu, 1972). The phylloplane of the plants is populated by the microorganisms included in the category of micromycetes, bacteria and actinomycetes. Between these microorganisms, there occur relationships of competition for space and food. These useful relations, which are designed to naturally keep pathogens in check, can be investigated and exploited for the establishment of biological control measures. Studies carried out by Romanian and foreign researchers have shown that application of different chemical treatments changes the composition of useful mycoflora that acts in

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phylloplane an antagonistic role (Fokkema, 1978; Drăgoescu, 1983; Oprea, 1987; Palaghiciuc, 2007).

The researches, conducted in 2009-2011 period in experimental plots of S.C. Hofigal Export-Import S.A. of Bucharest, aimed at identification and isolation of both some strains of pathogenic fungi of black currant crop, which could weaken the quality of the row material and some strains of saprophytic fungi which acting in the phyllosphera and which could be used for biological control of these currant pathogens. Alternative non-polluting control methods would be the only clean solution to obtain phytopharmaceutical products, with no toxicity for humans.

MATERIAL AND METHOD

The biological material was the plant organs of black currant shrubs in experimental plots of S.C. Hofigal S.A. in Bucharest. For the microscopic examination, there were collected in sterile sample bags the leaves, buds, branches, flowers or fruits, according to phenophase.

The vegetal organs that showed characteristic lessions were directly subjected to microscopic analysis. If the reproductive structures were not cured, the leaves or fragments of branches with visible signs of attack were kept at the wet room first for stimulation of spore formation and then examined under a microscope. Larger leaves or branches were cut into fragments. Buds, leaves, fragments of leaves and fruits were placed in Petri vessels on the surface of water-agar culture medium to stimulate sporulation, the method used by the Romanian researchers (Oprea, 1987; Palaghiciuc, 2007).

The petri plates were incubated at room temperature and after 10 days, they have been examined microscopically to identify resulting mycoflora. Isolation of fungi in leaf mycoflora was made by transfer under sterile conditions of spores on culture media rich in nutrients, such as PDA (potato-dextrose-agar) medium and MEA (malt-extract-agar) medium. For the identification of fungal genera and species, there have been used both macroscopic examination of fungal colonies and micoscopic examination methods.

RESULTS AND DISCUSSIONS

On the leaves, the evolution of the number of genera of fungal genera has varied in relation with the degree of leaf maturation (tab. 1).

In certain periods of development, after the microscopic examination, structures with a role in phytopatogenic fungi spreading, such as conidia and cleistothecia of the fungus *Sphaerotheca mors-uvae* (fig. 1d), conidia of *Alternaria tenuissima* (fig. 1o,p), picnidia with picnospores or perithecia with asci of fungus *Mycosphaerella grossulariae* (fig. 1h), conidia of *Gloeosporium ribis* (anamorph of the fungus *Drepanopeziza ribis*), uredospores and teleutospores of the patogen *Cronartium ribicola* (tab. 1).

Table 1

Evolution of the number of genera and species of mycromicetes during the vegetation period of the plants of *Ribes nigrum* L. under pedo-climatic conditions of Bucharest area

Micromicete	***Frequency***			
	at the first leaflet	***before flowering***	***at maturation of the leaves***	***on senescence***
MICROMICETE PARAZITE				
Sphaerotheca mors-uvae	+++	+++	+ *(conidia and perithecia)*	+ *(perithecia)*
Alternaria tenuissima	-	-	++ *(conidia)*	+
Mycosphaerella grossulariae	-	-	+++ *picnidia)*	++
Gloeosporium ribis	-	-	++ *(conidia)*	+++ *(perithecia)*
Cronartium ribicola	-	-	++ *(uredospoes)*	++ *(teleutospores)*
Botrytis cinerea	-	-	-	-
MICROMICETE SAPROFITE				
Trichoderma viride	-	++	+	+
Trichotecium roseum	-	++	++	++
Clonostachys rosea	-	+	+	+
Epicoccum nigrum	-	-	++	++
Chaetomium globosum	-	+	++	+
Alternaria spp.	+	++	+++	++
Alternaria alternata	+	++	+++	+++
Cephalosporium spp.	+	++	-	-
Cladosporium herbarum	-	++	++	+
Penicillium spp.	+	++	-	+
Penicillium frequentans	-	-	+	++
Fusarium oxysporum	-	+	++	+
Ulocladium spp.	-	-	+	+
Chladomyces spp.	-	+	+	-
Chaetomyces spp.	-	+	+	+
Pericornia spp.	-	+	+	+
Monospora spp.	-	+	+	+
Humicola grisea	-	++	+	-
Papularia spp.	-	+	-	++
Memnispora spp.	-	-	++	-

Legend: +++ high frequency
++ middle frequency
+ slight frequency

The first signs of American mildew fungus *Sphaerotheca mors-uvae* have appeared in spring at the first appearance of shoots of only formed leaves.

These leaves and small twigs remained were deformed and twisted and dried up. The brown mycelium with cleistothecia appeared in mid-June (fig. 1b, fig. 1c). Under strong attack, in 2009, the brown mycelium of *S. mors-uvae* with

cleistothecia was found on the mature fruit, which suffered from cracking and rotting on the parts affected (fig. 1a).

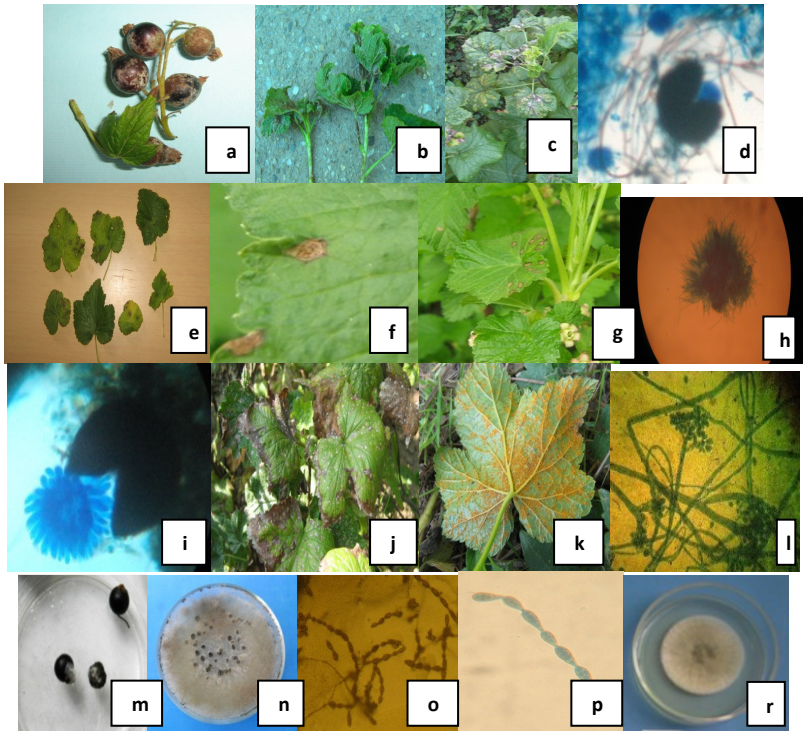

Fig. 1 – Pathogenic micromycetes of the black currant culture: macroscopic, microscopic and culture *in vitro* aspects

D. ribis was manifested on the leaves (fig. 1j). Since July, on the infected leaf blade, there appeared reddish brown spots, circular or irregular, at the beginning 1-2 mm diameter. Over time the stains were increased, becoming confluent and occupied a large portion of the leaf blade. In the spring 2010 in the plantation of S.C. Hofigal S.A., on the fallen leaves from the ground surface perithecia of fungus *M. grossulariae* containing asci with ascospores were observed (fig. 1i). In the second half of April, in the flowering phenophase, the first spots appeared on the leaves (fig. 1e, fig. 1f, fig. 1g). In the center of the spots picnidia with picnospores were identified (fig. 1h). The number of dots on the attacked leaves varied from 1 to 19 dots. To the end of vegetation period, the number of dots on the attacked leaves increased, on a leaf being even 200 dots. It was noticed the confluence of dots in certain cases, but also the phenomenon of the perforation of the leaves (fig. 1e). The fungus manifested with a high frequency in July and August months at maturation of the leaves (tab. 1).

Alternaria black spot disease, caused by the fungus *Alternaria tenuissima* (fig. 1o-r) appeared in June, on the leaves, as brown-gray spots with lighter edges.

Currant rust, produced by *Cronartium ribicola*, was observed in late summer, on the underside of the leaf blade as yellow-orange cylindrical columns of teleutospores (fig. 1k). Gray mold fungus *Botrytis cinerea* produced fruit rot. In high humidity, especially on the surface of fruits kept in the laboratory after harvest, there was a heavy gray coat (fig. 1l), composed of mycelium, conidiophores and conidia of fungus (fig.1n).

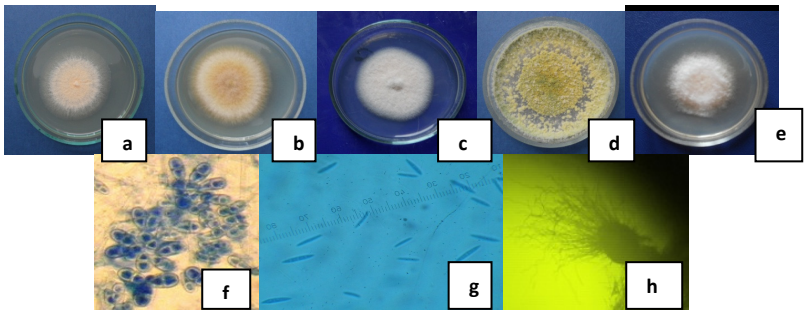

Fig 2 - Isolated saprophyte micromycetes of the phylloplan of the black currant culture: macroscopic, microscopic and culture *in vitro* aspects

Leaf mycoflora was dominated by saprophytic species, such as species of *Alternaria* spp, *Cladosporium herbarum*, *Penicillium* spp, *Fusarium oxysporum* (fig. 2g), *Cephalosporium spp.* Among the fungi known to act in phylloplane an antagonistic role against of plant pathogens of different crops (Şesan, 1997) have been identified and isolated saprophytic fungi (tab. 1) like *T. viridae* (fig. 2d)., *E. nigrum* (fig. 2b), *C. globosum* (fig. 2h), *Gliocladium roseum* (fig. 2c), *T. roseum* (fig. 2a, fig. 2f). The isolated of fungi were used to prepare a collection of potential antagonistic strains for to research the *in vitro* relations between them and the strains of pathogenic fungi in order to identify methods of biological control of pathogens. In leaves with no signs of attack, have been also identified fungi that develop in phylloplane toxins that could impair the quality of raw material and could damage of humans consuming products from these materials, such as *Alternaria* spp. producing alternariol, *Penicillium frequentans* frequentic acid producing (Hulea, 1995).

CONCLUSIONS

1. In the vegetation period, *Ribes nigrum* leaves were inhabited by fungi (micromycetes) and some species of bacteria and actinomycetes were also present;

2. Saprophytic micromycetes predominated, being represented by species of *Alternaria* spp., *Cladosporium herbarum*, *Penicillium* spp., *Fusarium oxysporum*, *Cephalosporium* spp.;

3. Saprophytic fungi have been developed with the antagonistic role in phylloplane as *T. viridae*, *E. nigrum, C. globosum*, *Clonostachys rosea*, *T. roseum* were isolated in order to establish a collection of fungi;

4. In phylloplane are present saprophytic fungal species that developing in phylloplane, which could alter quality of raw material and could damage humans consuming products obtained from these materials;

5. In certain periods of plant development were identified pathogenic fungi such as: *S. mors-uvae*, *A. tenuissima*, *M. grossulariae*, *D. ribis*, *C. ribicola*

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