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MICROFLORA ASOCIATED TO RHIZOSPHERE AND RHIZOPLANE OF GRAPEVINES IN SOME ROMANIAN WINE-GROWING AREAS

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ABSTRACT

In this paper is presented a study on microorganisms isolated from rhizosphere of grapevines located in Arad County (Mini wine-growing areas) and Prahova County (Valea Călugărească wine-growing areas). Given that these areas were chosen for the experimental application of organic fertilizer microbiologically enriched, the purpose of this work was to identify the natural occurrence of microorganisms that inhabit the grapevine rhizosphere and rhizoplane. The micromycetes isolated from the rhizosphere were mostly saprophytic; some of them have antagonistic role and 7% of rhizoplane are pathogenic fungal microorganisms. The rhizosphere is also colonized by bacteria, actinomycetes and nematodes.

Key words: *microflora, rhizosphere, rhizoplane, grapevine*

INTRODUCTION

Soil serves as a suitable medium for development of microorganisms, a fact illustrated by their very high number and diversity. Soil contains microorganism populations with very different biological and biochemical characteristics: bacteria, fungi, algae and protozoa. Identification of microorganisms is both theoretically and practically important for assessing their role in ecosystem functioning.

The phenomenon of accumulation of microorganisms around the root zone is well known and was broad reported. The biological composition of microbial communities and the ability of some microorganisms to colonize the rhizosphere of grapevine are known to play important role in plant health. In plant protection studies, there are presented results on the interactions of micro flora in the root zone due to the effect of phytosanitary products.

Abroad, it was published papers mainly concerning the bacterial associated with roots and rhizosphere of grapevines. In Argentina it was published a study on bacterial strains isolated at different soil depths from roots and rhizosphere of grapevines from a commercial

vineyard (Salomon et al., 2013). Dore (2009) published an extensive study on the influence of grapevine rhizosphere bacteria on some root diseases. Aballay (2011) presented a screening of rhizosphere bacteria from grapevine for their suppressive effect on *Xiphinema index.*

Fungi from the rhizosphere and rhizoplane were analyzed in grape vine *Vitis labrusca* (de Lima, 2014).

In the context of using biological means for protection and nutrition of grapevine, the present study is justified by the fact that the effectiveness of the fertilization treatments using microbiologically enriched fertilizers depends on the interaction between microorganisms that naturally colonize the grapevine rhizosphere/rhizoplane and the beneficial microorganisms from the fertilizers composition.

The purpose of this study was to determine the microbiota in relation to the rhizosphere and rhizoplane of some Romanian grapevine (*Vitis vinifera),* by morphological identification of microorganisms.

MATERIALS AND METHODS

Portion of the soil that is influenced by the root system (rhizosphere samples) and residue of soil adherent to the root surface (rhizoplane samples) from wine-growing areas (figure 1) - Miniş (Arad county) and Valea Călugărească (Prahova county) - were analyzed according to the method of soil analysis described by Clark (1965). Soil samples were collected from depths of 30, 60 and 90 cm. The samples were stored in the refrigerator at 4°C, until examination.

Direct isolation method was carried out for pathogenic, saprophytic and antagonistic fungal microorganisms (figure 2). One gram was weighed from each sample, suspended in 9 ml of sterile distilled water with TW80 (0.01%) and then distributed in Petri dishes, on water agar medium, using several decimal dilutions. From each dilution, 0.5 ml suspension was distributed with Drigalsky spatula. The plates were incubated at room temperature (22 ° C) for 12 days. Semi-selective medium Dodine – PDAY (Dodine Supelco, 0.1g/l, penicillin-G Sigma 0.4g/l, streptomycin sulphate Sigma Ald. 1g/l, yeast extract Difco 1g/l, PDA Fluka 39g/l) was used for isolation of enthomopathogenic fungus.

Soil washing method was used for removing spores and conidia of abundant sporulating species, in order to isolate fungal mycelia of fungi that grow slowly or are found within the organic particles. One gram of soil was suspended in 200 ml sterile water, shaken

for 15 minutes and allowed to settle at a 45° angle for 10 minutes. The operation has been repeated several times, until the remaining particles dispersed in water. Few dilutions were made for obtaining approximately 25 fungal colonies on agarized medium in Petri dishes.

Identification of the microorganisms was made according to Barnett (1960), Samson (1981), Booth (1971), Raper and Fennell (1965), Ramirez (1982) and Ellis (1960).

RESULTS AND DISCUSSION

The results concerning the natural incidence of microorganisms revealed that the rhizosphere and the rhizoplane of grapevines from analyzed sites were colonized by different types of microorganisms: bacteria (24%), fungi (12%), actinomycetes (64%).Of these 36% were pathogenic species.

The results regarding microorganisms isolated from Miniş grape vine are shown in table 1.Biological material derived from Valea Călugărească presented a rootstock with thin roots and fray on the bark (figure 1).

Figure 1. Rhizosphere/rhizoplane soil samples and vine roots collected for the microbiological analysis

The soil from the rhizosphere was populated by bacteria, actinomycetes and micromycetes (table 2). Isolate of *Gonatobotrys simplex* was obtained, which is a myparasite on *Alternaria alternata (*syn. *tenuis)*, a plant pathogen (Hoch, 1977). Fungus able to degrade some herbicides and fungicides, *Cunninghamella elegans* (Hangler, 2007 ; Jairaj,1997; Pothuluri,1993; Zhu, 2010) was also identified in the rhizosphere samples.

The frequency of micromycetes and actinomycetes (*Actinomyces* and *Streptomyces*) was 71% and 21%, respectively. Compared to fungus, bacteria were isolated in smaller proportion (8%). The micromycetes inhabiting the rhizosphere are mostly saprophytes, some of them having antagonistic role. Of these, 7% are pathogenic in rhizoplane: *Verticillium dahliae*, *Cylindrocarpon destructans*, *Fusarium sambucinum* enter into plant tissues through naturally or artificially produced wounds (by insects, mechanical works, etc.), causing rot and death of the plant. It was also reported the presence of nematodes.

CONCLUSIONS

The rhizosphere of vine plants was populated by bacteria, fungi and actinomycetes. Observations led to the conclusion that following factors have influenced the rhizosphere colonization: soil organic substances, their depth distribution and the humidity. The microroganisms have developed in relation to the distribution of organic substance and depth (soil moisture).

Roots of vine plants from Miniş were almost entirely damaged. Bacteria (12%), actinomycetes (24%) and fungi (64%) have developed in the root stock rhizosphere. Nematodes were also present.

Vine plants from Valea Călugărească showed thin roots, fray and damaged bark. The rhizosphere has also reported the presence of bacteria (8%), actinomycetes (21%) and micromycetes (71%). In rhizosphere, fungi belonging to the genera *Verticillium*, *Cylindrocarpon*, *Fusarium* and *Melanospora* were commonly found in all samples analyzed.

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Table 1. Microorganisms isolated from rhizosphere of vines from Miniş

Sample nb. (rootstock)	Isolated microorganisms	Behavior
1 rootstock rhizosphere	*Actynomicetes* ++	saprophytic
	Streptomyces++	pathogenic
	Bacteria +	saprophytic
	Gliocladium roseum ++	antagonistic
	Gliomastix murorum+	antagonistic
	Cephalosporium mycophilum+	pathogenic
	Lasiobolus pulcherrimus++	saprophytic
	Cylindrocarpon destructans+	pathogen on roots
	Mucor.+	saprophytic
	Mortierella sp.+	saprophytic
2 rootstock rhizosphere	*Actynomicetes*++	saprophytic
	Streptomyces+	pathogenic
	Bacteria ++	saprophytic
	Candida sp.+	saprophytic
	Absidia glauca+	saprophytic
	Cunninghamella elegans++	saprophytic
	Periconia sp.	saprophytic
	Trichoderma viride++	antagonistic
	Verticillium chlamidosporium+	pathogen on roots
	Melanospora fallax ++	pathogen on roots
	Micromonospora+	saprophytic
	Acreomonium butyri i++	pathogenic
3 rootstock rhizosphere	*Bacteria* ++	saprophytic
	Actynomicetes++	saprophytic
	Streptomyces+	pathogenic
	Absidia glauca++	saprophytic
	Mortierella sp.+	saprophytic
	Cephalosporium sp.+	antagonistic
	Periconia sp.+	saprophytic
	Scopulariopsis brevicaulis+	antagonistic
	Paecelomyces lilacinum++	antagonistic
	Doratomyces++	saprophytic
	Gliocladium roseum++	antagonistic
	Penicillium frecquentans+	saprophytic
	Lasiobolus pulcherrimus ++	saprophytic
	Papulaspora imersa	saprophytic

+ = low frequency, ++ = average frequency, +++ = strong frequency

Table 2. Microorganisms isolated from rhizosphere of vines from Valea Călugărească

Sampling zone/ depth of soil sample	Isolated microorganisms	Behavior
(1)	(2)	(3)
(zone I) 5 – 120cm	*Actinomyces*++	saprophytic
	Streptomyces+	pathogenic
	Penicillium granulatum++	saprophytic
	Gliocladium roseum++	antagonistic
	Cephalosporium mycophilum+	antagonistic
	Verticillium dahliae++	pathogen on roots
	Periconia++	saprophytic
	Gonatobotrys simplex +	antagonistic
	Gliomastix murorum+	antagonistic
	Trichoderma viride++	antagonistic
	Alternaria tenuissima+	pathogenic
	Ulocladium consortiale++	saprophytic
	Cunninghamella elegans+	saprophytic
	Candida sp.++	saprophytic
(zone II) 5 – 100 cm	*Actinomyces*++	saprophytic
	Streptomyces+	pathogenic
	Mortierella sp.+	saprophytic
	Acreomonium butyri +	antagonistic
	Scopulariopsis brevicaulis+	antagonistic
	Gliomastix murorum++	saprophytic
	Gliocladium roseun +	antagonistic
	Trichoderma viride++	antagonistic
	Penicillium frequentans+	saprophytic
	Penicillium janthinellum+	saprophytic
	Lasiobolus pulcherrimus+	saprophytic
(zone III) 50 cm	*Bacteria*+	saprophytic
	Candida sp.+	saprophytic
	Paecilomyces variotii++	Antagonistic
	Monodictis sp.+	Saprophytic
	Acreomonium butyri +	Antagonistic
	Mortierella sp.+	Saprophytic
	Gliomastix murorum++	Antagonistic
	Gliomastix cerealis++	Antagonistic
	Humicola grisea+	Saprophytic
	Cylindrocarpon radicicola+	pathogen on roots
(zone IV) 30cm	*Penicillium janthinellum*+	Saprophytic
	Mortierella hyalina+	Saprophytic
	Humicola grisea+	Saprophytic
	Arthrobotrys oligospora++	Nematicide
	Verticillium dahliae++	Pathogenic
	Gliomastix murorum+	Antagonistic
	Fusarium sambucinum+	Pathogenic
	Candida sp+	Saprophytic
	Monocillum mucidum+	decompose wood
	Lasiobolus pulcherrimus+	Saprophytic
	Phoma exigua+	Pathogenic

continued table

(1)	(2)	(3)
(zone V) 90 cm	*Penicillium janthinellum*+	saprophytic
	Lasiobolus pulcherrimus+	saprophytic
	Gliocladium roseum+	antagonistic
	Verticillium dahliae+	pathogenic
	Periconia sp.+	saprophytic
	Acreomonium butyri +	saprophytic
	Oidiodendron+	saprophytic
	Cuninghamella+	saprophytic
(zone VI) 60 cm	*Actinomycetes*+	saprophytic
	Acreomonium butyri	saprophytic
	Verticillium dahliae++	pathogenic
	Absidia sp.+	saprophytic
	Mortierella++	saprophytic
(zone VII) 90 cm	*Actinomycetes* ++	saprophytic
	Verticillium dahliae ++	pathogenic
	Cuninghamella elegans +	saprophytic
	Lasiobolus pulcherrimus +	saprophytic
(zone VIII) 30 cm	*Acreomonium butyri* +	saprophytic
(zone IX) 60 cm	*Acreomonium butyri* +	saprophytic
	Cuninghamella elegans +	saprophytic
	Gliocladium roseum+	antagonistic
	Arthroderma uncinatum+	keratinophilic species
	Dactilaria+	saprophytic
	Verticillium dahliae ++	pathogenic
(zone X) 90 cm	*Actinomyces* +++	saprophytic
	Streptomyces ++	pathogenic
	Verticillium dahliae ++	pathogenic
	Doratomyces+	saprophytic
	Penicillium frequentans	saprophytic
	Alternaria tenuissima+	pathogenic

+ = low frequency, ++ = average frequency, +++ = strong frequency