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FUNGI INHABITING DECAYING GRAPEVINE (*VITIS* SPP.) CUTTINGS

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Abstract: The purpose of the study, carried out in the years 2001–2003 was to determine which fungal species inhabited decaying grapevine cuttings during callusing and soon after planting them into pots. The plant material was collected from 5 commercial plantations and 8 cultivars, which were most frequently cultivated. From each plantation and cultivar 20 cuttings with symptoms of the growth inhibition or decay were randomly sampled during the callusing period i.e. March/April (term I) and 2–3 months after planting the cuttings into pots i.e. June/July (term II). The results showed that from affected grapevine cuttings *Phomopsis viticola, Botrytis cinerea, Alternaria alternata* and *Fusarium* spp. were isolated most frequently. Moreover, it was found that after planting young cuttings into the pots, numerous isolates of soil borne pathogenes were obtained, among others *Cylindrocarpon* spp., *Phytophthora* sp., *Rhizoctonia solani*, *Fusarium* spp.

Key words: grapevine, cuttings, fungi

INTRODUCTION

The study conducted in the recent years in the Polish grapevine nurseries revealed that every year from 8 to 30% of potted grapevine cuttings decayed lub showed decay symptoms. The decay can be attributed to various biotic and abiotic factors, including viruses, bacteria and fungi (Pearson and Goheen 1998; Theron and Crous 1998; Halleen et al. 2003). Usually, grapevine cuttings are infected with commonly occurring, polyphagous soil borne pathogens as *Cylindrocarpon* spp., *Fusarium oxysporum*, *Phytophthora* spp. and *Rhizoctonia solani* after planting in nurseries (Marais 1979; Scheck et al. 1998; Omer et al. 1999; Halleen et al. 2003). Moreover, a lot of fungal species inhabit the above-ground parts of grapevine, including shoots and might be commonly widespread with mother canes on the new plantations (Mungai et al. 1999; Péros et al. 1999; Mostert et al. 2000). Among numerous pathogens *Phaeoacremonium* spp. causing Petri disease has been recognized as a major cause of the decay of young grapevines (Theron and Crous 1998; Mugnai et al. 1999; Halleen et al. 2003). Significant dam-

age of grapevine cuttings is also attributed to other fungal species such as *Phomopsis viticola* Sacc., *Eutypa lata* (Pers, Fr.) Tul. et c. Tul. and *Botryosphaeria* spp. causing various nacrotic lesions, inhibition of growth and consequently decay of diseased plants (Philips 1998; Mugnai et al.1999; Péros et al. 1999; Mostert et al. 2000; Castillo-Pando et al. 2001; Król 2006).

The Polish literature lacks any information about fungi associated with decay of grapevine cuttings and simultaneously colonised mother canes. The study conducted in the recent years indicated that healthy grapevine canes grown for vegetative reproduction are inhabited, by several fungal species and some among them are regarded as pathogenic towards grapevine, like *Phomopsis viticola*, *Botrytis cinerea*, *Phoma negriana* (Machowicz-Stefaniak 1993; Machowicz-Stefaniak and Kuropatwa 1993, 1993a; Machowicz-Stefaniak and Król 2006; Król 2006).

The aim of the present study was to determine which fungal species inhabited decaying grapevine cuttings, with special reference to phyllosphere pathogens, and to establish their occurrence in some nurseries.

MATERIALS AND METHODS

The survey conducted in the years 2001–2003 included 5 commercial plantations and 8 cultivars of grapevine which were the most frequently cultivated: Agat Donskij, Bianca, Gołubok, Iza Zaliwska, Muscat Letnij, Prim, RF-16 and Schuyler (Król 2006). The plant material was collected from one chosen nursery in Podkarpackie, Mazowieckie and Wielkopolskie provinces and from two nurseries in Lubelskie province. Majority of cuttings (from 4 plantations inspected) were cultivated under cover, at the same conditions. Each time 20 cuttings with symptoms of inhabited growth or decay were sampled in two terms, i.e. March/ April – during callusing (term I) and July/June – 2–3 months after planting the cuttings in the pots (term II). For each term of study totally 2400 diseased cuttings were collected (5 plantations x 8 cultivars x 20 cuttings x 3 years). Mycological analyses of cuttings were carried out in laboratory using artificial culture method (Machowicz-Stefaniak and Kuropatwa 1993). Strips of tissue from all length of callused graft and young, green shoots (term I) and in case of inhabited cuttings additionally from young roots (term II) were collected for fungi isolation. The plant material was superficially disinfected for 2 min. in 10% sodium hypochlorite and washed 3 times for 3 min. in sterile distilled water and next, cut into 3 4-mm pieces of tissue and plated in Petri dishes with 2% potato-dextrose agar (PDA, bioMérieux). For each combination of the experiment 100 pieces of tissue were used. The Petri dishes with plant material were incubated in a thermostat at 22°C for 10 days. Growing fungal colonies were transferred to PDA slants for further identification.

RESULTS

Mycological analyses showed that 35 fungal species were obtained from affected cuttings (Table 1). The number and species composition of fungi isolated from the shoots of decaying cuttings were similar at both dates but they slightly differed from the species composition of fungi isolated from the roots (Table 1). For instance *P. viticola* invaded shoots of 230 cuttings in term I and 227 ones in term II, and only the roots of 2 examined cuttings. Fungi from the genera *Alternaria alternata* and *Bortytis*

cinerea were also most frequently isolated from the shoots of callusing cuttings (term I) and the rooting ones (term II); than from the roots (Table 1). The number of shoots affected with these fungi ranged from 93 in the case of *B. cinerea* to 270 for *A. alternata* (Table 1). Besides, it was observed that fungi from genus *Gloeosporium ampelophagum, Phoma* spp., *Cytospora* spp. and saccharomycetes were obtained only from the shoots of vine cuttings (Table 1).

Table1. Fungi isolated from decaying grapevine cuttings in the years 2001–2003

Fungal species	No. of colonised cuttings (out of 2400 inspected) and no. of (isolates obtained)		
	Shoots		Roots
	Term 1	Term 2	Term 2
Acremonium charticola (Lindau.) W. Gams	6 (10)	21 (37)	25 (55)
Acremonium kilienze comb. nov.		14 (34)	
Aureobasidium pullulans (de Bary) Arnold	17 (39)	28 (47)	7 (12)
Alternaria alternata (Fr.) Keissler	233 (634)	270 (470)	35 (70)
Aspergillus sp.		20 (32)	37 (58)
Botrytis cinerea Pers.	93 (209)	104 (229)	15 (29)
Chaetomium globosum Kunze	37 (60)	41 (83)	40 (78)
Cladopsorium cladosporioides (Fres.) de Vries.	50 (78)	36 (53)	15 (27)
Cylindrocarpon destructans (Zins) Scholten	11 (25)	19 (45)	49 (116)
Cylindrocarpon obtusisporum (Cooke et Harkness) Wollenw.	9 (21)	35 (69)	84 (209)
Cytospora sp.	27 (58)	10 (18)	
Epicoccum purpurascens Ehrenb.	43 (87)	33 (59)	14 (42)
Fusarium avenaceum (Fr.) Sacc.	26 (63)	11 (17)	5 (7)
Fusarium culmorum (W.G.Smith) Sacc.	2 (3)		
Fusarium equiseti (Corda.) Sacc.	4 (7)	10 (17)	24 (51)
Fusarium oxysporum Schlecht. emend. Snyd. et Hans	55 (134)	72 (201)	102 (273)
Fusarium sambucinum Fuckel	27 (40)	16 (35)	20 (53)
Fusarium semitectum Berk et Rav.	14 (42)	23 (45	11 (21)
Fusarium solani (Mart.) Appel et Wollenw. emend	172 (515)	209 (637)	186 (598)
Fusarium sporotrichioides Sherb.	26 (61)	15 (43)	14 (40)
Gliocladium catenulatum Gilman et Abbott	152 (321)	159 (283)	93 (152)
Gliocladium fimbriatum Gilman et Abbott	73 (46)	52 (97)	42 (79)
Gliocladium roseum Bainier	37 (87)	40 (56)	
Gloeosporium ampelophagum (Pass.) Sacc.	8 (17)	16 (33)	
Phoma herbarum (Pers.) Link	29 (45)	18 (30)	
Phoma negriana Thümen	14 (22)	16 (23)	
Phomopsis viticola Sacc.	230 (495)	227 (505)	2 (2)
Penicillium spp.	17 (38)	37 (66)	25 (49)
Phytophthora sp.			22 (56)
Trichoderma harzianum Rifai	36 (86)	38 (87)	26 (70)
Trichoderma koningii Oud.	84 (200)	65 (168)	45 (121)
Trichoderma viride Pers. ex Gray	3 (6)		
Trichothecium roseum (Pers.) Link	3 (6)	25 (48)	
Rhizoctonia solani Kühn	49 (96)	68 (143)	135 (349)
Yeast		17 (28)	
Total	(3651)	(3738)	(2617)

Numerous isolates of *Fusarium* spp. and less numerous of *Gliocladium* spp. and *Trichoderma* spp. were always obtained from the shoots and the roots. The fungi lised above were obtained from 326, 262 and 123 shoots of diseased cuttings at term I, and from 356, 251 and 71 ones at term II, respectively (Table 1). The same species were obtained from the roots of 362, 135 and 71 cuttings, respectively (Table 1). It was found out that *F. oxysporum* and *F. solani* most frequently colonised grapevine cuttings among the numerously isolated other species from genus *Fusarium* (Table 1). In addition, *Cylindrocarpon* spp. and *Rhizoctonia solani* were also isolated from the inspected shoots and roots. These species inhabited mainly the roots since they were isolated from 133 and 135 cuttings, respectively while the isolates obtained from the shoots originated from 20 and 49 cuttings in term I and from 54 and 68 cuttings in term II, respectively (Table1).

The studies also revealed that the isolates of *Phytophthora* sp. were obtained only from the roots of the decaying cuttings (Table1).

The other fungal species occurred with low frequency and the number of cuttings affected with them was from 2 for *F. culmorum* to 43 in the case of *E. purpurascens* (Table 1).

DISCUSSION

It seems that in Polish conditions *P. viticola* is the most dangerous pathogen of grapevine shoots causing the decay of cuttings at callusing and soon after their planting. The common isolation of this fungus from diseased cuttings recorded in presented here studies as well as previously published data have proved ability of *P. viticola* to infect grapevine shoots in the climatic conditions of Poland (Machowicz-Stefaniak 1993; Machowicz-Stefaniak and Kuropatwa 1993) and also its capability of latent development in the shoots of this plants (Król 2006). Similar results describing the harmfulness of *P. viticola* towards grapevine cuttings in various regions of the world were published earlier (Pearson and Goheen 1988; Scheper et al. 1997; Mostert et al. 2000). Supposedly the production of grapevine cuttings in Poland mainly under covers creates conditions of high temperature and humidity favourable for the development of *P.viticola* (Pearson and Goheen 1988; Machowicz-Stefaniak 1993; Machowicz-Stefaniak and Kuropatwa 1993) i.e. facilitating the infection and development of pathogen in the tissues of the diseased plants. Similar frequency of isolating *P. viticola* from the shoots of callusing cuttings and soon after their planting to pots suggests that the mother canes are main source of young plants infection.

Among the other species isolated from the shoots of the decaying cuttings, *B. cinerea* should be also considered harmful to grapevine (Machowicz-Stefaniak and Kuropatwa 1993a: Mostert et al. 2000; Halleen et al. 2003) It is known that this fungus can be the primary pathogen causing the spots and decay of berries, while the ability for the saprophytic mode of life makes it possible for its to survive in the plant's environment and also to infect the shoots if proper conditions would appear (Mostert et al. 2000; Halleen et al. 2003). Moreover, the results indicate the possibility of transplanting of some fungi species inhabiting mother canes into the cuttings (Król 2006), what agrees with other researches (Pearson and Goheen 1988; Scheper et al. 1997; Mostert et al. 2000; Halleen et al. 2003).

Common isolation of numerous species from the genus of *Fusarium* calls for further explanation of their effect on the healthiness of grapevine cuttings although other authors suggest that their importance in the plants' phyllosphere is small despite

the fact that they are often isolated (Halleen et al. 2003). However, it is known from the literature that *F. oxysporum* inhabiting the soil environment can cause the decay of grapevine cuttings (Highet and Nair 1995; Omer 1999). Isolating *Phytophthora* spp. and majority of isolates of *Rhizoctania solani* and *Cylindrocarpon* spp. only from the roots confirms the opinion according to which infection comes from the soil environment (Marais 1979; Scheck et al. 1998; Halleen et al. 2003).

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POLISH SUMMARY

GRZYBY IZOLOWANE Z ZAMIERAJĄCYCH SADZONEK WINOROŚLI (*VITIS* SPP.)

Celem badań prowadzonych w latach 2001–2003 było określenie gatunków grzybów zasiedlających zamierające sadzonki winorośli, ze szczególnym uwzględnieniem gatunków fylosferowych. Do badań wytypowano 5 szkółek produkcyjnych, w różnych regionach Polski i 8 najczęściej uprawianych odmian. Z każdej plantacji i odmiany analizowano po 20 sadzonek z objawami zahamowania wzrostu lub zamierania. Próby pobierano w czasie kalusowania, tj. marzec/kwiecień (termin I) oraz po 2–3 miesiącach od wysadzenia roślin, tj. czerwiec/lipiec (termin II). Wykazano, że pędy zamierających sadzonek winorośli zasiedlały najczęściej *Phomopsis viticola, Botrytis cinerea, Alternaria alternata* i *Fusarium* spp. Ustalono, że łozy mateczne są głównym źródłem infekcji młodych roślin. Z korzeni badanych sadzonek uzyskiwano liczne gatunki przeżywające w glebie, m.in. *Cylindrocarpon* spp., *Phythophthora* sp., *Rhizoctonia solani*, *Fusarium oxysporum* i *F. solani*.