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The evaluation of grape vine decline pathogens in the experimental field of the Georgikon Faculty of Agriculture in Cserszegtomaj

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Summaryt: Vine decline causes important economic loss in viticulture, especially in longer view. Several causal pathogen were described lately, however little is known about the etiology or epidemiology of these pathogens on grapevine rootstock. It is well known that these diseases affect grafted and rooted grapevines and are not specific to any scion-rootstock combinations. Our aim was to determine what pathogens are presents in the experimental field, especially causal agents of the rootstock decline. Different grapevine rootstocks and scion varieties were tested in our trial. Isolations were made from the wood tissue and pathogenity tests were done with isolated *Cylindrocarpon destructans*. The possibility of infection during the propagation process was studied as well. Most commonly *Cylindrocarpon sp.* and *Phomopsis sp.* species were identified from the examined varieties. *Cylindrocarpon destructans* was able to spread to apical (shoot) and basal (root) direction from the point of infection with uneven speed. Callus development is not inhibited by the fungi causing the leaf symptom of the vine decline. Shoot development is reduced if unhealthy parts are grafted to each other.

Key words: vine decline, trunk diseases, *Cylindrocarpon* sp., rootstocks

Introduction

One of the most important factors for economically successful viticulture is to have a healthy, complete vineyard without missing plants. However some of the vine-stocks became dead before it should happen by the natural biological process. Generally, little more than the halves of the vineyards have more than 6% of missing plants in Hungary.

Vine decline is one of the most increasing problems in our vineyard nowadays. A group of parasitic fungi ('decline pathogens') cause direct damage through attack on the xylem tissue. More than 80 species of fungi listed as responsible casual agent of vine decline in the international literature. The following fungi are the most often cultured among them: *Phaeomoniella chlamydospora, Phaeoacremonium* sp. (*Mugnai* et al. 1999), *Cylindrocarpon sp., Fomitiporia punctata* (*Cortesi* et al. 2000), *Eutypa lata* (*Rolhausen* et al. 2006), *Phomopsis viticola, Botryosphaeria spp,* (*Úrbez Torres* et al. 2006).

Different methods are applied for the identification of the responsible, agent of the disease in Hungary and in other countries as well. Regarding the rootstock vine decline very little information is available of the pathogens believed to be involved. Grape propagation materials are well mentioned, including rootstocks, as one of the source to spread the disease in vineyards.

The aims of our research have been to determine which fungi are causing the symptoms in the experimental field, to identify the casual organism of the disease, study the possibility of infection, to develop a management strategy to prevent or cure these plants, which are showing the typical symptoms of vine decline.

Material and method

The experiments were established in the first decade of September 2003. Vine stocks of *Vitis vinifera* L. cultivars and grape rootstock varieties *(1 table)* were chosen.

Table 1 The used cultivars for the field survey

Number	Species, Cultivars
1	Rip x Rup 101–14
2	Georgikon 28
3	Ber. X Rip (T. 4A SO4)
4	Ruggeri 140
5	V. sol. x rip. (1616)
6	Rup du Lot
7	Mendeleum
8	Teleki F. SO4 K 39
9	Rubics Tajsovsky
10	*Vitis vinifera cv.* Nektár, Zöld vertelini, Cserszegi fűszeres, Chardonnay

Figure 1 A vinestock which is showing the typical symptoms of fungal vine decline

We identified the typical foliar of vine decline (*Figure 1*) and we marked the vines with different colored flags in the experimental field of the University of Pannonia, Georgikon Faculty of Agriculture in Cserszegtomaj (46°48'22''N, 17°13'52''E).

The wood samples arms, canes; shoots were collected from the chosen vines.

Isolations were made from the wood tissue. During the isolation work we chose the discolored areas of the tissue. According to *Cortesi* et al. (2000) methods we made the isolation from the wood tissue.

The surface of the samples was disinfected in 90% Ethanol (Sigma-Aldrich Budapest, Hungary). After the disinfection the surface of the tissue was cut away to expose the discolored area. Small pieces of the discolored wood tissue were placed on 9 cm diameter Petri dishes (Spektrum3D ltd. Budapest, Hungary) containing 4% of potato dextrose agar (PDA) (Sigma-Aldrich Budapest, Hungary). Cultures were incubated on room temperature until fungal colonies were observed. The fungal colonies were identified according to their morphological characteristics.

The spread of the isolated *Cylindrocarpon destructans*, originated from the variety was evaluated on *V. berlandieri* x *V. Riparia* Teleki SO4, *V. berlandieri x V. rupestris* Ruggeri 140 and *Vitis solonis* x *V. riparia* 1616 C rootstocks during pathogenicity test. Two bud section of rootstock canes originated from the experimental farm of the University were rooted under controlled environment (24 °C, 80% RH) in glasshouse condition.

One month was enough to get rooted them. At first we established a clean plate culture of the fungus on PDA and in the next step we artificially infected the rootlings. The open wound on the stem was covered with parafilm (American National Can, Chicago IL.) to keep the moisture and avoid any other infection.

The aim of the other experiment was to study the possibility of infection in the grafting components during the propagation process. The experiment was established in the first decade of January 2004. It had four combinations using canes from the observed varieties from the experimental field (infected) (S) vinestock and rootstock and also using canes from symptomless (H) scion with symptomless rootstock combination. We observed the callus formation after 9 months and we made isolations from the scion and the rootstock.

Results and discussion

Isolations from the field

Most of the infected vinestocks (56 pieces) according to the morphological characteristics of the colonies were carrier of *Cylindrocarpon destuctans* Zins.(syn: *Cylinrocarpon radicicola* Wollenw.), *Fusarium solani.*, *Verticillium sp.* and *Alternaria sp.* and we also isolated canker pathogens. Canker symptoms were caused by *Botryosphaeria obtusa (Schwein.)*, *Phomopsis viticola (Sacc.)*. Most of the symptoms were associated with black foot disease. Most commonly *Cylindrocarpon sp.* and *Phomopsis sp.* species were identified, mainly *Cylindrocarpon destuctans*. It was the first isolation of *Cylindrocarpon destructans* in Hungary from grapevine rootstock.

Two types of *Cylindrocarpon destructans* were distinguished culturing them on PDA media according to their different color. There were no differences between the pathogenicity of the different isolates. The size of the conidias were measured and compared with the literature (*Vörös* et. al. 1965).

Pathogenicity test

During the pathogenicity test, we were able to isolate and identify the fungus *Cylindrocarpon destructans* in apical and basal directions from the wound (20–60 mm)

Table 2 Callus formation differences between each grafting type

	Grafting combinations			
	H–H*	H–S	S–S	S–H
Callus formation percentage	92%	62%	78%	66%
Shoot development	75%	30%	0%	23%

*H means healthy, S means symptomatic. The first letter always marks the rootstock, and the second letter marks the scion in the combinations.

Figure 2 The shoot development after 2 month in the combination H-H

Figure 3 The shoot development after 2 month in the combination H-S, S-H

after two months. We have stated from our experiment that the fungus was able to spread to apical (shoot) and basal (root) direction from the point of infection with uneven speed. It has moved faster to basal direction than to apical.

First the percentage of the callus formation was observed during the grafting experiments (*Table 2*). Callus formation was evaluated after 3 weeks of callusing according to the methods of *Kocsis & Bakonyi* (1994).

All the combinations produced callus tissue at grafting place and on the basal site as well. It means that the infection does not prohibit the callus formation of the scion or rootstocks. The best results were given by the symptomless scion with symptomless rootstock combination (H-H). If we have examined the shoot development we observed that none viable graftings were resulted with the infected

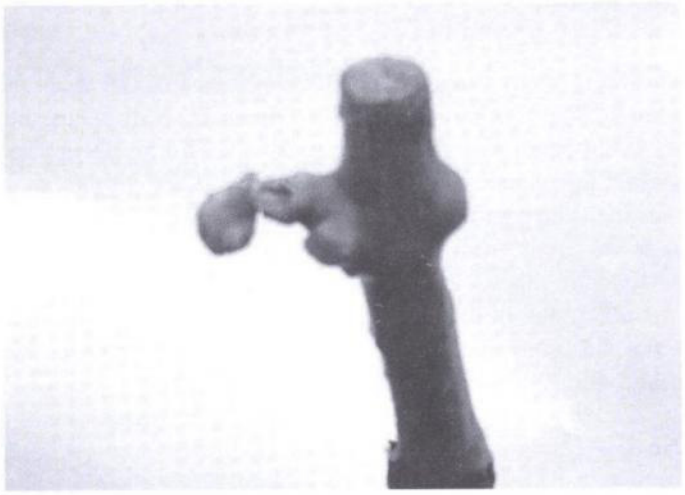

Figure 4 The shoot development after 2 month in the combination S-S

(symptomatic) scion grafted on infected rootstock. They have produced callus tissue, but none of them were able to develop shoot. If symptomless scion was grafted on infected rootstock or infected scion was grafted on symptomless rootstock grafting was viable. The shoot development was less in vigor of these combinations to compare the healthy graftings and the grafting percentage was lower as well (*Figure 5*). No leaf symptoms were noted on that time of the year (*Figure 2, 3 and 4*).

The plants were examined at the end of the vegetative cycle, at the end of September. The plants were cut 100-150 mm below the graft scare and longitudinal section was made of each. We were able to note the spread of the disease from the point of grafting with measure the necrotic spot of the tissues on the longitudinal section. The fungi were capable to get into the healthy part of the plant for example the symptomless rootstocks get infected with the infected scion from the top. We determined that most of the dead tissue occurred at the spot of the grafting and the opposite site of the bud formation. We could isolate *Cylindrocarpon destructans* from the discolored section.

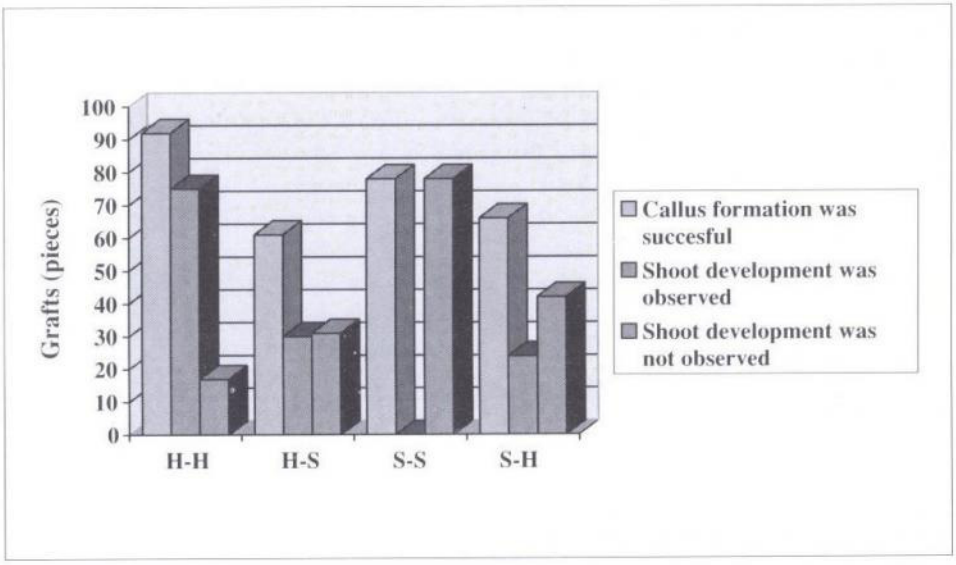

Figure 5 The differences in the shoot development between the grafts

Discussion

Out of many fungi what s mentioned as a causal agent of vine decline *Cylindrocarpon* and *Phompsis* seems to us the most important regarding to the rootstock. Our results is very similar to compare Rego et al. (2000) where both of the fungi were isolated commonly from rootstocks in Portugal, however it is different from other results where more commonly Phaeocremonium and Phaemoniella were isolated but fromscion part of the stocks. We have obtained two different color isolations of *Cylindrocarpon destructans*, however we didn't find differences between their pathogenicty.

Callus development is not inhibited by the fungi causing the leaf symptom of the vine decline. However the cell differentiation could not happen without difficulties because shoot development was reduced if unhealthy parts were grafted to each other.

Our study shows the disease is able to get into the healthy plant during the grafting process. It remains symptomless during the nursery production; we didn't observe any leaf symptoms. But in the wood tissue the fungus was able to grow during the vegetation. This way the pathogens could spread to new vineyards by grafting.

We hypothesized that the infection occurs from the soil in our experimental planting. Therefore two strategies could be applied to prevent spreading the causal agents via rootstocks a., avoid to use the basal part of the canes for grafting procedure; b., avoid getting too much wounds on the plants and treat with chemical or biological products.

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