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FUNGI ASSOCIATED WITH ESCA DECLINE AND THEIR *IN VITRO* CONTROL BY CHITOSAN

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Abstract

Esca is a devastating and insidious disease that affects vineyards in major grape-producing areas worldwide. Several esca characteristics make the effectiveness of control strategies still difficult (types of microorganisms involved, correlation of foliar symptoms with wood deterioration).

The aims of our research were to identify fungal pathogens associated with esca decline and to evaluate their in vitro control by chitosan. Research was carried out in Bucharest (N $44^{0}25^{'}$, E $26^{0}6^{'}$) in 2009, in a plantation founded in 1994 of Feteasca regala cultivar grafted on Kobber 5 BB. The type of vine training system can be a favourable factor for esca decline. Both, the mild and the severe form of esca were observed. Samples obtained from vines with characteristic esca symptoms were examined for the presence of pathogenic fungi. The fungi isolated from the wood were included in the genera: Phaeoacremonium, Phaeomoniella, Phomopsis, Fomitiporia, Fusarium, Alternaria, Cladosporium, Aspergillus and Botryosphaeria.

In vitro assays investigated the influence of chitosan on the mycelial growth of esca pathogens. The effective concentrations which inhibit mycelia growth by 90% were calculated. Our results highlighted the potential of chitosan to control esca pathogens. Further investigation is needed to set an integrated management program in which chitosan could be used as wound dressing.

INTRODUCTION

Esca is a devastating and insidious disease that affects vineyards in major grape-producing areas worldwide. The esca diagnosis is based on external symptoms on leaves, and internal degradation of wood tissues. Several esca characteristics make the effectiveness of control strategies still more difficult: a) several fungi have been associated with the disease; b) in most cases, foliar symptoms shows up when wood discoloration and decay inside the vine are already extensive; c) foliar symptoms vary on the same vine from year to year - it appears to be some relationship between fungal development in the host plant and climatic parameters as air temperature, rainfall [5].

With no chemical control available for treatment of grapevine trunk diseases, management options are largely preventive and limited to minimizing infection risk by pruning in dry weather whenever possible.

The aims of our research were to identify fungal pathogens associated with esca decline and to evaluate their *in vitro* control by chitosan (poly b-(1-4)N-acetyl-d-glucosamine), a deacetylated form of chitin, and known as a natural antimicrobial compound.

MATERIAL AND METHODS

Research was carried out in Bucharest (N 44^0 25', E $26^0$6') in 2009, in a plantation founded in 1994 of Feteasca regala cultivar grafted on Kobber 5 BB. Vines were spaced 2.20 x 1.20 m by using three pruning systems (Guyot on demi-high trunk, Cazenave cordon and spur-pruned cordon).

Plant material. Samples obtained from vines (Feteasca regala cultivar) with characteristic esca symptoms were examined for the presence of pathogenic fungi. Samples were composed by canes (vine shoots), larger branches (arms) and trunks of vines of Feteasca regala cultivars with variable symptoms of disease.

Fungal isolation. Plant materials were surface sterilized (soaked in 10% bleach for five seconds), dipped in sterile distilled water, and dried on sterile filter paper. The samples were cut and cultured on Malt-Agar (MA) plates (2% + 2% w/v) for the recovery of the pathogens. Cultures were incubated at 25ºC in the dark. Fungal colonies were transferred to MA plates, incubated at room temperature and identified according to their morphological characteristics (morphology of colonies, the microscopic characteristics of mycelium, conidiophores, and conidia).

Antifungal activity. Antifungal activity of chitosan (Sigma Chemicals) on 6 esca fungal isolates was tested *in vitro*. Mycelial discs (8 mm in diameter) were cut from the margin of colonies and placed, mycelium downwards, in Petri dishes containing MA and chitosan (0.0625 - 10.125 - 0.25 - 0.5 - 1 - 2%). Chitosan stock solutions were prepared [2]. All plates were incubated at 25^0C in the dark. Colony diameters were measured and the percentage of inhibition of radial growth (values are the means of three replicates) was calculated. Results are expressed as effective concentration EC50 (the concentration which reduced mycelial growth by 50%).

RESULTS AND DISCUSSION

Esca decline was typically identified in the mild form, as symptoms on leaves: interveinal regions of chlorotic and yellowish tissue that turns yellow-brown or red-brown (Figure 1a). The severe form (dieback of one or more shoots, leaf drop, shriveling and drying of fruit clusters - Figure 1b) was also observed. Both form of esca were observed on 5% of the Feteasca regala cultivar. In cross sections, the most common dieback symptoms were: a) internal wood decay: white rot in the centre of the trunk, soft, friable wood mass; b) central necrosis, brown wood (Figure 2); c) wedge-shaped lesions were the least common.

The type of vine training system can be a favourable factor - the mode of pruning can favor disease spread. Esca decline was found in only 2.4% of vines trained by Cazenave cordon, in 3.7% of vines trained by Guyot on demi-high trunk but in 13.58% vines trained by Spur-pruned cordon. In vines trained by multiple Guyot and Guyot with periodically replaced arms, esca decline was found in 6.79, respectively 7.4% (Figure 3).

Fungal pathogens associated with esca decline. Nine fungal species were isolated from the wood of the grapevines with esca decline symptoms. The isolates were identified according to their morphological characteristics on potato dextrose agar. The fungi isolated from the wood were included in the genera: *Phaeoacremonium, Phaeomoniella, Phomopsis, Fomitiporia, Fusarium, Alternaria, Cladosporium, Aspergillus* and *Botryosphaeria*.

The fungal species detected in trunks with esca decline, and the frequencies at which they occurred are shown in Figure 4. *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* are recognized as the primary colonizing agents in the development of esca in older vines [3, 4]. An infection involving the *Phaeoacremonium* and *Phaeomoniella* species predisposes the vines to wood rots caused by basidiomycete fungi such as *Fomitiporia punctata.* In the last stage of wood degradation, the two fungi are frequently associated with other pathogens, in particular *Fomitiporia* sp. isolated from the white-rotted wood.

In vitro effect of chitosan on mycelial growth of esca fungal pathogens. Chitosan, a deacetylated form of chitin, is a natural biodegradable compound derived from crustaceous shells. *In vitro* assays investigated the toxic effects of chitosan on the mycelial growth of esca pathogens. The effective concentrations which inhibit mycelia growth by 90% were calculated. For all tested isolates, the mycelial growth was strongly affected by chitosan, with complete inhibition starting to 0.25% (Table 1). Our results highlighted the potential of chitosan to control esca pathogens.

Chitosan has been proven to control numerous pre- and postharvest diseases on various horticultural commodities [1]. In addition to its direct microbial activity, it is strongly suggest that chitosan induces a series of defense reactions.

Due to its ability to form a semi permeable coating, could be use as wound dressing. Further investigation is needed to set an integrated management program in which chitosan could be used as wound dressing.

Table 1

Sensitivity of esca isolates to chitosan

Isolate	Code	EC50 [%]
Phaeoacremonium chlamydosporum	Pc 1205	0.20
Phaeomoniella aleophilum	Pa 809	0.12
Fusarium sp.	F 205	0.10
Aspergillus niger	An 305	0.20
Alternaria alternata	Aa 1704	0.10
Cladosporium sp.	C 542	0.15
Botryosphaeria dothidea	Bd 2320	0.20

a. b.

Fig. 1. Esca foliar and symptoms (a); shriveling and drying of fruit clusters (b)

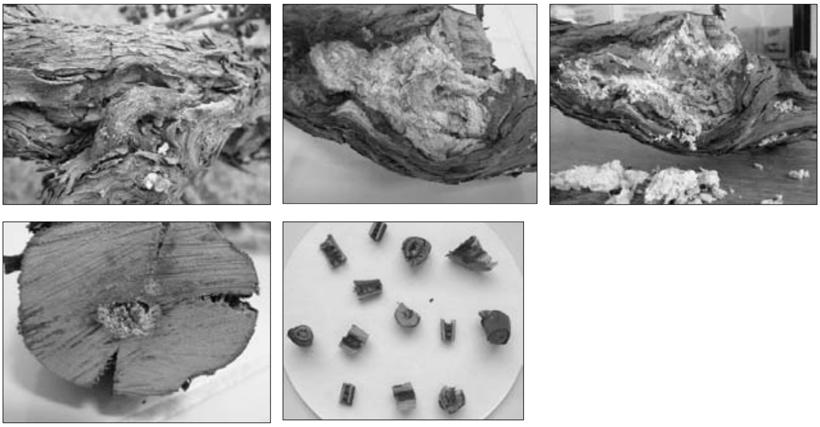

Fig. 2. Cracking of a grapevine trunk. Internal wood decay
(white rot, friable mass; central necrosis, brown wood)

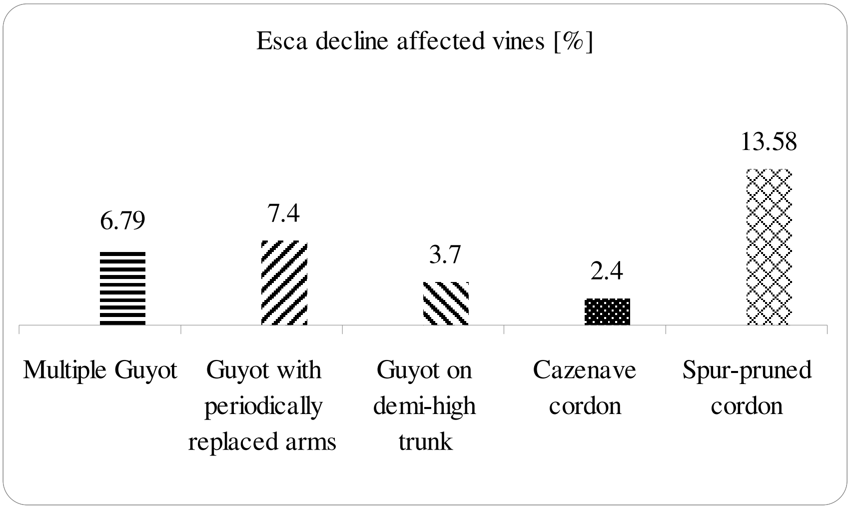

Fig. 3. Type of vines training and frequency of esca decline

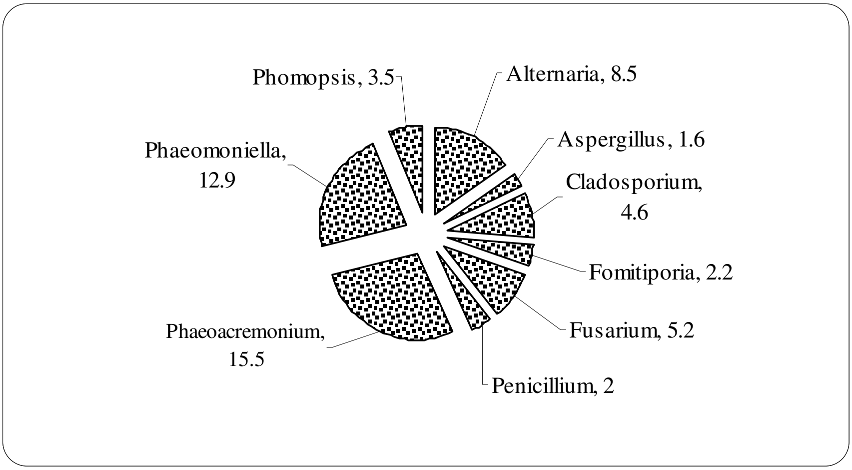

Fig. 4. Frequency of fungal species associated with esca decline

CONCLUSIONS

1. Our survey confirmed the presence of esca decline, typically identified in the mild and the severe form on vines of the Feteasca regala cultivar. The type of vine training system can be a favourable factor. Esca decline was found in only 2.4% of vines trained by Cazenave cordon but in 13.58% vines trained by Spur-pruned cordon.
2. In symptomatic vines, nine fungal species were isolated from the wood of the grapevines with esca decline symptoms. The fungi isolated from the wood were included in the genera: *Phaeoacremonium, Phaeomoniella, Phomopsis, Fomitiporia, Fusarium*, *Alternaria, Aspergillus, Cladosporium* and *Botryosphaeria*.
3. Our results highlighted the potential of chitosan to control esca pathogens. Further investigation is needed to set an integrated management program in which chitosan could be used as wound dressing.

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