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Phaeoacremonium chlamydosporum and *Phaeoacremonium angustius* associated with esca and grapevine decline in *Vinho Verde* grapevines in northwest Portugal

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Summary. *Vinho Verde* is a unique wine, exclusively produced in the northwest region of Portugal. The production of this wine is an important component of portuguese agriculture. The aims of the present work were to make a preliminary assessment of the presence of esca and grapevine decline in the *Vinho Verde* region, and to check for the presence of *Phaeoacremonium* spp. in diseased plants. The results showed that esca and grapevine decline do occur in the *Vinho Verde* region, and can be widespread in this region. From ten diseased grapevines we isolated nine strains of *P. chlamydosporum*, and one strain as *P. angustius*. Our results agree with other reports that *P. chlamydosporum* is frequently isolated from the wood of grapevines showing symptoms of esca or grapevine decline. *P. aleophilum* and *P. inflatipes* were not isolated from the plants examined in the present study. Instead, *P. angustius* was isolated from a diseased plant.

Key words: esca, Portugal, *Phaeoacremonium*.

Introduction

Esca and grapevine decline are two of the most destructive diseases of the woody tissues in grapevine. The etiology of these diseases is still partly unknown. Several different fungi have been implicated, and among these are *Phaeoacremonium chlamydosporum* and *Phaeoacremonium aleophilum*. These fungi are determinant in the infection process as pathogens, since they have been isolated with high frequency from the wood of diseased vines, can cause disease in experimentally inoculated plants, and are able to colonize living wood *in vitro* (Ferreira *et al.*, 1994; Mugnai *et al.*, 1996; 1999; Calzarano and Di Marco, 1997; Larignon and Dubos, 1997; Scheck *et al.*, 1998a; 1998b; Surico *et al.*, 1998; Ferreira, 1999; Pascoe, 1999).

Vinho Verde is a unique wine, exclusively produced in the northwest region of Portugal (Fig. 1). It is the most important agricultural activity of this region, where 90% of the farmers produce *Vinho Verde*. The production of this wine is an important component of Portuguese agriculture and economy. The area occupied by *Vinho Verde* grapevines is 16% of the total national grapevine area. The amount of *Vinho Verde* produced (more than

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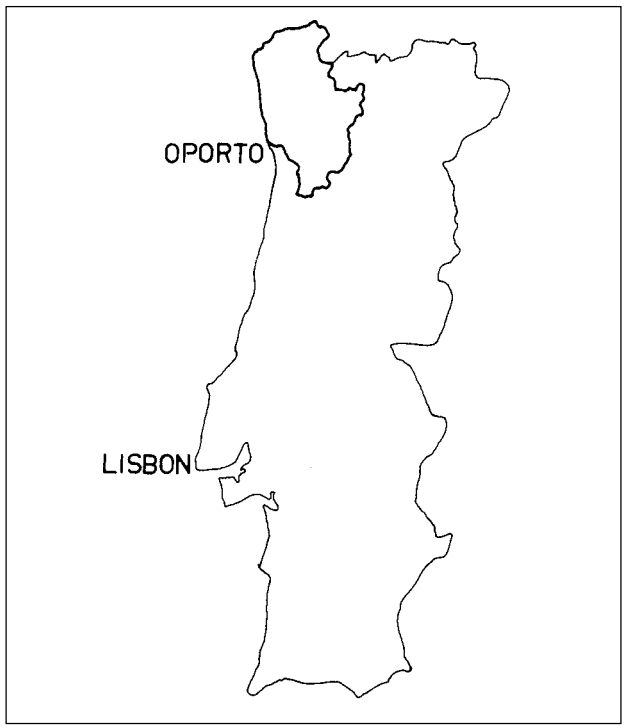

Fig. 1. Map of Portugal showing the region of *Vinho Verde*.

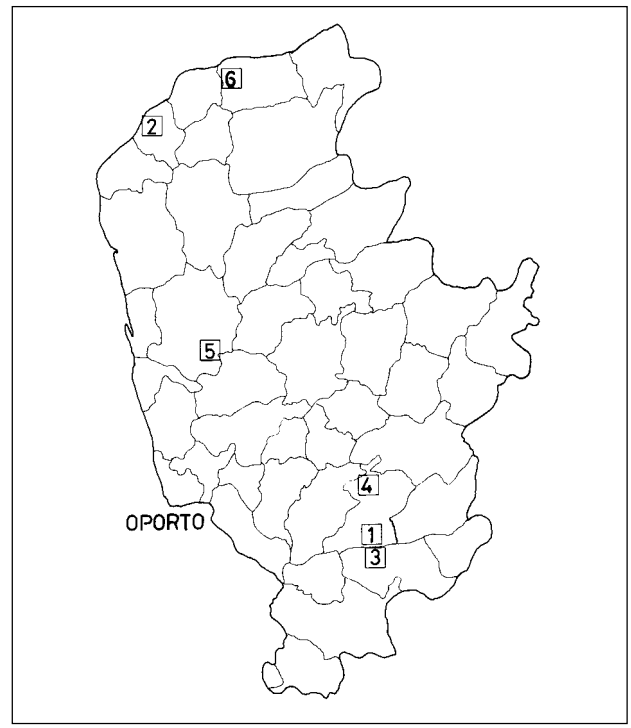

Fig. 2. Map of the region of *Vinho Verde* with the locations of sampling sites.

1,000,000 hl per year in 1994-1998) is 17% of the total national wine production (Anonymous, 1994-1998). The main cultivars for white wine are: 'Alvarinho', 'Avesso', 'Azal Branco', 'Loureiro', 'Pedernã' and 'Trajadura' (Carvalho, 1997).

Esca and grapevine decline have been reported from central and southern Portugal (Rego *et al.*, 1999). The aims of the present work were to make a preliminary assessment of the presence of these diseases in the *Vinho Verde* region, and to check for the presence of *Phaeoacremonium* spp. in diseased plants.

Table 1. Sources of strains recovered from diseased vines.

Location		Rootstock/cultivar (age)	Symptoms in the wood	Tissues cultured	Fungal strains code number
Concelho	Code number Figure 2				
Marco de Canavezes	1	?/Avesso (7 y)	Black spots	From rootstock	EA II (b)
Marco de Canavezes	1	?/Avesso (7 y)	Central brown necrosis	From rootstock	EA II (a) 1
Vila Nova da Cerveira	2	41B/ungrafted (1 y)	No symptoms	From rootstock	A III
Cinfães	3	196-17/Avesso (5 y)	Black spots	From cultivar	CF VI
Cinfães	3	196-17/Avesso (5 y)	Black spots	From rootstock	CF VIII
Marco de Canavezes	4	196-17/Avesso (3 y)	Black spots	From rootstock	REG 72 III
Barcelos	5	?/Pedernã (11 y)	Black spots	From cultivar	REG 117 III
Barcelos	5	?/Pedernã (11 y)	Black spots	From cultivar	REG 117 V
Monção	6	?/Alvarinho (23 y)	Central brown necrosis bordered by a black line	From cultivar	QB I
Marco de Canavezes	1	?/Avesso (7 y)	Central brown necrosis	From rootstock	EA II (a)

Table 2. Main characteristics and identification of the fungal strains.

Strain code number	Presence of allantoid conidia	Presence of chlamydospores	Radial growth rate (mm/d)	Colour of the colonies (back)	Identification
EA II (b)	-	+	25.5ºC: 1.6 30.5ºC: 0.46 35ºC : 0	green-gray	*P. chlamydosporum*
EA II (a)	1 + (14%)	-	25.5ºC: 1.6 30.5ºC: 1.7 35ºC : 0	dark-brown with yellow pigment	*P. angustius*
A III	-	+	25.5ºC: 1.6 30.5ºC: 1.2 35ºC : 0	green-gray	*P. chlamydosporum*
CF VI	-	+	25.5ºC: 1.7 30.5ºC: 0.67 35ºC : 0	green-gray	*P. chlamydosporum*
CF VIII	-	+	25.5ºC: 1.4 30.5ºC: 0.45 35ºC : 0	green-gray	*P. chlamydosporum*
REG 72 III	-	+	25.5ºC: 1.5 30.5ºC: 0.40 35ºC : 0	green-gray	*P. chlamydosporum*
REG 117 III	-	+	25.5ºC: 1.8 30.5ºC: 0.32 35ºC : 0	green-gray	*P. chlamydosporum*
REG 117 V	-	+	25.5ºC: 1.5 30.5ºC: 0.30 35ºC : 0	green-gray	*P. chlamydosporum*
QB I	-	+	25.5ºC: 1.6 30.5ºC: 0.62 35ºC : 0	green-gray	*P. chlamydosporum*
EA II (a)	-	+	25.5ºC: 1.6 30.5ºC: 0.45 35ºC : 0	green-gray	*P. chlamydosporum*

Materials and methods

Isolation

Ten diseased grapevines of different cultivars and rootstocks from various vineyards in the region were used in the present study. Details are given in Table 1 and Figure 2. Cross sections of the woody stem of the rootstock and cultivar of each vine were examined. Small pieces (*ca.* 3x2x2 mm) of necrotic tissue were placed in Petri dishes containing potato dextrose agar (Difco). Cultures were incubated in the dark at 25ºC.

Table 3. Dimension of conidia in *Phaeoacremonium chlamydosporum* and *P. angustius* strains.

Strain code number	Number of spores measured	Length (μm)			Width (μm)			Length/ width
		mean	range	median	mean	range	median	
EA II (b)	326	3.9	2.6-6.1	3.9	1.8	1.1-2.6	1.8	2.2
EA II (a) 1	343	5.2	3.4-7.9	5.0	2.0	1.3-7.1	2.1	2.6
A III	175	4.0	2.9-6.3	3.9	1.8	1.3-2.6	1.8	2.3
CF VI	298	3.7	2.4-5.3	3.7	1.8	1.3-2.9	1.8	2.0
CF VIII	124	4.1	2.9-6.1	4.2	2.1	1.3-2.9	2.1	2.0
REG 72 III	178	3.6	2.1-5.3	3.4	1.5	0.8-2.4	1.4	2.5
REG 117 III	175	3.5	1.8-8.4	3.4	1.6	1.1-2.4	1.6	2.2
REG 117 V	292	3.6	1.8-9.2	3.5	1.6	0.8-2.4	1.6	2.3
QB I	329	3.6	2.4-5.5	3.4	2.1	1.3-2.9	2.1	1.7
EA II (a)	303	3.5	2.5-5.0	3.4	2.0	1.3-3.2	1.8	1.8

Characterization of the isolates

Isolates were grown on 2% Difco malt extract agar (MA). Dimensions of conidia were determined by the following procedure. Conidia harvested from young cultures (less than 7-day-old) and suspended in distilled water, were mounted on glass slides and photographed at random using a light microscope and color film. The slides were enlarged and the cells drawn with a pencil on white paper. An objective micrometer (Reichart, Germany), photographed and magnified in the same conditions as the conidia, was used to calculate the exact magnification, which was 1,900 x. The length and width of each conidium was determined by direct measurement of the drawing with a ruler, followed by division by magnification. The number of measured conidia is shown in Table 3. Radial growth rates were determined by using the following procedure. Agar plugs (4 mm diam.) from the margin of young cultures were placed at the center of MA plates, and incubated at 25.5, 30.5 and 35°C, in the dark (with three replicate plates of each culture at each temperature). The colony diameters were measured at intervals, and, plotted against time, gave a straight line. Radial growth rate was determined by linear regression analysis, using the least-squares method.

Results

Occurrence and symptoms

In the period 1994-1999, we observed esca and grapevine decline causing serious losses in wine production in 32 medium- and large-sized farms of *Vinho Verde* all over the region. From 138 grapevines received in our laboratory with fungal infections, 67 had typical symptoms of esca or grapevine decline. All cultivars of *Vinho Verde* grapevines were affected.

The old grapevines showed brown necrosis in the woody stem bordered by a thick black line, cracks along the trunk, chlorotic foliage, and dark spots on the berries (Fig. 3). The young grapevines showed stunted growth, brown-black wood-streaking with gummosis, and in the leaves, interveinal chlorosis, marginal necrosis and wilting (Fig. 4 and 5).

Characterization of the isolates

Ten isolates had the following characteristics: hyphae septate, hyaline and simple, and becoming light brown and forming strands in old cultures; conidiogenous cells phialidic, solitary, subcylindrical or swollen, smooth; conidia aggregated into round, slimy heads at the apices of the phialides; conidia aseptate, hyaline, cylindrical to ellipsoid, mostly straight. These strains were identified as belonging to the genus *Phaeoacremonium* (Crous *et al.*, 1996).

Nine strains had the following characteristics (Tables 2 and 3). Colonies (reverse) green-gray. Abundant production of chlamydospores. Swollen phialides. Conidia cylindrical to ellipsoid, straight, with mean length and width ranging from 3.5 to 4.1 and 1.5 to 2.1 μm, respectively. Radial growth rate decreased in the order 25.5,

Fig. 3. An old grapevine with symptoms of esca showing dark spots on the berries and chlorotic foliage.

Fig. 4. Young plants with grapevine decline showing stunted growth and chlorotic foliage.

Fig. 5. Longitudinal section of the wood of a young plant with grapevine decline – the rotted wood is bordered by brown streaking.

30.5, 35°C. These cultures were identified as *P. chlamydosporum* (Crous *et al.*, 1996), although conidia mean width exceeded the range of dimensions reported by Crous *et al.* (1996).

One strain had the following characteristics (Tables 2 and 3). Colonies (reverse) dark brown with a diffusing yellow pigment. Absence of chlamydospores. Conidia cylindrical to ellipsoid, mostly straight (less than 1/5 allantoid), with mean length and width of 5.2 and 2.0 μm, respectively. Conidia could be divided in two populations (Figure 6): most of conidia were short (the population was centered at 4.8 μm), and a small fraction of the cells was long (the population was centered at 6.6 μm). Allantoid conidia occurred in both populations. Radial growth rate decreased in the order 30.5, 25.5, 35°C. This culture was identified as *P. angustius* (Crous *et al.*, 1996), although the optimum growth temperature was not 25°C as reported by these authors, but near 30.5°C.

Other characteristics of the isolates are given in Tables 2 and 3.

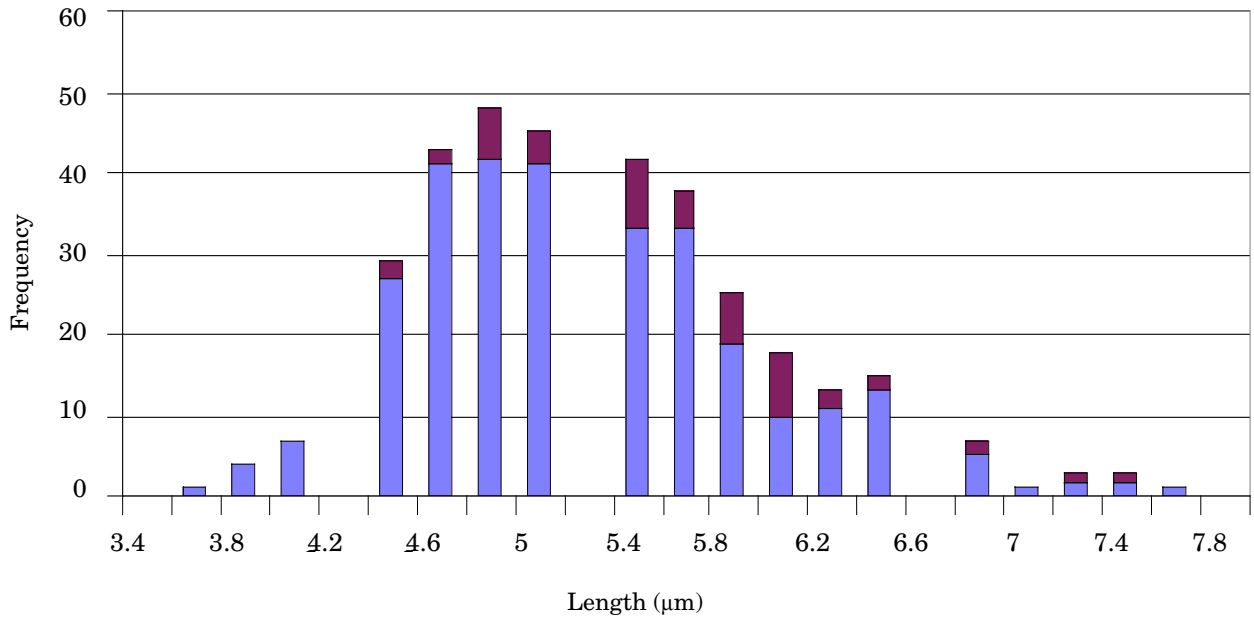

Fig. 6. Histogram of conidia length of strain EA II (a) 1 (*P. angustius*). Upper part of the bar, allantoid conidia; lower part of the bar, straight conidia.

Discussion

Esca and grapevine decline have been reported in central and southern Portugal (Rego *et al.*, this issue). The results presented in this work show that these diseases also occur in the northwest, in *Vinho Verde* grapevines, and can be widespread in this region. Considering the economic importance of *Vinho Verde* production, the occurrence of esca and grapevine decline in *Vinho Verde* region is of great concern and deserves further research.

Our results agree with other reports that *P. chlamydosporum* is frequently isolated from the wood of grapevines showing symptoms of esca or grapevine decline (Ferreira *et al.*, 1994; Mugnai *et al.*, 1996, 1999; Calzarano and Di Marco, 1997; Larignon and Dubos, 1997; Scheck *et al.*, 1998a, 1998b; Surico *et al.*, 1998; Ferreira, 1999; Pascoe, 1999). *P. aleophilum* and *P. inflatipes* were not isolated from the plants examined in the present study. Instead we isolated *P. angustius* from a diseased grapevine. The isolation of this species from diseased grapevines have been seldom reported in the literature, and its role in esca and grapevine decline is unknown.

We isolate *Phaeoacremonium* not only from diseased grafted vines, but also from rootstock vines, as reported by Pascoe (1999). Considering the possibility that infection of young vines may originate from the rootstock (Ferreira, 1999; Mugnai *et al.*, 1999; Pascoe, 1999), the presence of these fungi in

Table 4. Dimension of conidia of *P. chlamydosporum* and *P. angustius*. Comparison between our results and those reported in the literature.

Species	Mean length (µm)			Mean width (µm)		
	This work	Crous *et al.* (1996)	Larignon and Dubos (1997)	This work	Crous *et al.* (1996)	Larignon and Dubos (1997)
P. chlamydosporum	3.5-4.1	3.0-4.0	2.4-6.0	1.5-2.1	1.0-1.5	1-2
P. angustius	5.1-5.3	4.5-6.0	Not reported	1.9-2.0	1.5-2.0	Not reported

rootstock vines in the *Vinho Verde* region should be thoroughly surveyed.

The dimensions of conidia and optimum growth temperature are very important characteristics in the delimitation of species in the genus *Phaeoacremonium* (Crous *et al.*, 1996). Our results concerning the dimensions of conidia in *P. chlamydosporum* agree with the values reported by Larignon and Dubos (1997), which are slightly different from the values reported by Crous *et al.* (1996) (Table 4). According to Crous *et al.* (1996) the optimum growth temperature of *P. angustius* is 25ºC. However, our results indicate an higher optimum temperature, *ca.* 30ºC. Although these differences can be related to the methods used, it appears that more studies are needed in order to clarify the taxonomy in the genus *Phaeoacremonium*.

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