

Isolation and Identification of fungi associated with Esca in Vinho Verde Region (RDVV) – northwest Portugal.

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INTRODUCTION

In the Portuguese *Vinho Verde* region (RDVV), during the last years the esca incidence is taking serious proportions. Several studies on esca and related syndromes have been carried out in this region. However, no study has ever been done to assess the extent of diversity within the esca fungi species isolated. The present study is a first step towards defining the morphological variation of the population of *Phaeoaniella chlamydospora* and *Phaeoacremonium inflatipes* in RDVV and in Portugal. The morphological, cultural and sporulation characteristics were used to determine the level of morphological heterogeneity in a representative sample of RDVV isolates of *Pa. chlamydospora* and *Pm. inflatipes*.

MATERIALS AND METHODS

Plant material - The study was carried out in the summers of 2004 and 2005 in three vineyards composed by 20-25 years old plants of *Vitis vinifera* cv. Alvarinho, located at the RDVV. Two were located in the Monção sub-region – Agra (A), Brejoira (B) – and one outside – in the Sousa sub-region – Lousada (L). Cordons were taken from 10 infected Alvarinho cultivar grapevines showing esca foliar symptomatology.

Isolation of fungi and morphological identification - In each vineyard selected, cordons were taken from 10 infected Alvarinho cultivar grapevines showing esca foliar symptomatology. Twenty five tissue pieces (ca. 3x2x2 mm) per plant were placed in 2% malt extract agar (MEA) in Petri dishes and incubated at 25°C in the dark. The colonies that developed were transferred to potato dextrose agar (PDA) for identification. Isolates were identified to species level by their morphological, cultural and sporulation characteristics (Crous et al., 1996; Crous & Gams, 2000; Mostert et al., 2006).

Statistic Analyse – The results obtained for the fungi isolation and morphological identification were underwent a hierarchical clustering analyse – group average method without scaling [NCSS software – PASS 2002 (J. Hintze, Statistical Systems, Kaysville, Utah, USA)].

RESULTS

Table 1: Fungi species and correspondent number of isolations in each vineyard selected.

Vineyard	Species	Isolations
Agra	<i>P. angustius</i>	3
	<i>P. chlamydospora</i>	7
	<i>P. inflatipes</i>	2
Brejoira	<i>P. chlamydospora</i>	9
	<i>P. inflatipes</i>	6
Lousada	<i>P. chlamydospora</i>	5
	<i>P. inflatipes</i>	9
	<i>P. viticola</i>	2

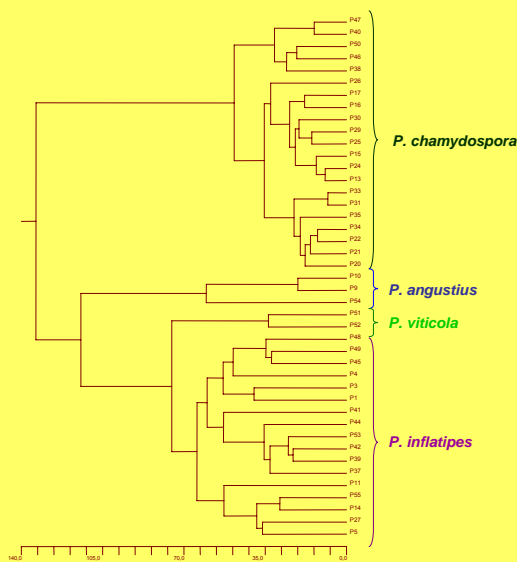


Figure 1: Cluster analysis of strains isolated in the three vineyards selected. Each strain is indicated by an abbreviation of the number of isolation. Bottom scale, dissimilarity.

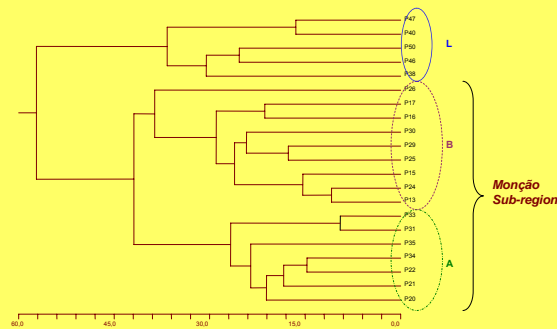


Figure 2: Cluster analysis of *Phaeoaniella chlamydospora* strains isolated in the three vineyards selected (Monção sub-region: A – Agra, B – Brejoira; Sousa sub-region: L – Lousada). Each strain is indicated by an abbreviation of the number of isolation. Bottom scale, dissimilarity.

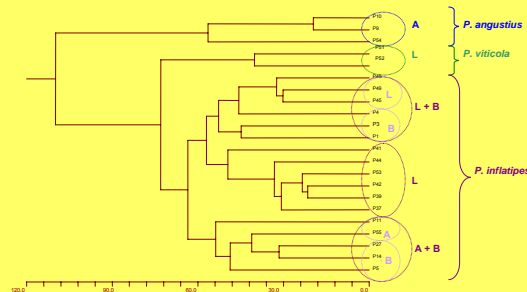


Figure 3: Cluster analysis of *Phaeoacremonium inflatipes* strains isolated in the three vineyards selected (Monção sub-region: A – Agra, B – Brejoira; Sousa sub-region: L – Lousada). Each strain is indicated by an abbreviation of the number of isolation. Bottom scale, dissimilarity.



MAIN CONCLUSIONS

► The isolation results confirm the occurrence of the species *Pa. chlamydospora* and *Pm. angustius* in RDVV, and identify for the first time the species *Pm. viticola* and *Pm. inflatipes*, both in this wine region and in Alvarinho grape vine variety.

► The results shows a high incidence of the species *Pa. chlamydospora* and *Pm. inflatipes* in RDVV. This is probably due to the fact that: these species naturally occur in vineyards soils and in grapevine tissues (Rooney et al., 2001); and that the specie *Pa. chlamydospora* is one of the primary pathogens associated to esca-diseased grapevines (Mugnai et al., 1999).

► Results show that there are different strains within one fungus specie in the majority of the isolates species, and also that fungi strains isolated from vineyards located in the same region have higher similarity.

► This preliminary work suggest that the regions environmental conditions where the grapevines are placed determine the morphological and cultural characteristics of the esca associated fungi species.

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